Resource Distribution in Ant Colonies

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RESOURCE DISTRIBUTION IN ANT COLONIES

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Declaration

None of the work in this thesis has been incorporated from a submission for any other degree. The colonies of ants were collected by Nigel Franks, Ana Sendova-Franks, Richard James and Tom Richardson; the experiments, marking of the ants and initial trophallaxis data collection were done by students Benjamin Wulf and Thomas Klimek of the University of Applied Sciences Bremen, supervised by Ana Sendova-Franks and Nigel Franks. Save for this, the work is my own.

Summary

The distribution of resources is vital to any system or society. This is particularly true of social insect colonies where independent access to resources is not available to all members. Only a fraction of individuals are responsible for obtaining food for the colony from outside the nest. Surprisingly little is known about how this food is subsequently distributed to members inside the nest. The work in this thesis is focused around a set of food distribution experiments conducted using four colonies of the ant *Temnothorax albipennis*. The study applies a well-used technique in a new way to investigate the distribution of food under two different scenarios: feeding under normal conditions and famine relief feeding after a period of starvation. All ants in each colony are marked and then individually tracked recording every feeding interaction to obtain a complete network of food transmission. This work has shown that all four colonies efficiently relieved the famine within 30 minutes of introducing new food. This process was facilitated by workers abandoning their spatial structure and expanding their space use; feeding multiple recipients from a single donor; and simultaneously spreading stored food and new food. Recruitment of foragers did not play a major role in relieving the famine but foragers were responsible for most of the first round of feeding. The study revealed that not all members received the same amount of food and most ants received food in multiple feeding interactions. The transmission pathways used to distribute the food present an opportunity for harmful substances to spread. The pathways are explored in this context to see whether the colonies might aim to minimize the spread by partitioning the pathways or maximise spread by mixing to promote social immunity. The study reveals behavioural differences between the four colonies which are likely to result from the inherent variation in demographic and geometric properties. These differences highlight the flexibility of ant colonies during problem solving under different conditions.

Chapter 1

Introduction

1.1 General introduction

The distribution of resources is vital to any system or society but must be achieved without simultaneously spreading harmful agents. Using ants as a model this thesis aims to explore how systems distribute resources to their constituent agents rapidly in a time of need. Ants are ideal for investigating optimization problems and processes such as resource distribution for several reasons. An ant colony can be viewed as a system of cooperative autonomous agents which exhibit simple probabilistic stimulus response behaviour resulting in complex spatio-temporal patterns at the colony level [1]. Ants have evolved over millions of years during which they have been able to select for optimal solutions which are flexible and robust, ultimately protecting the queen.

In this discovery based project the organisation of liquid food transmission among the workers in four colonies of the ant *Temnothorax albipennis* is explored under two scenarios: when food is abundant, "Control"; and when food is re-introduced after a period of starvation, "Famine Relief". The transmission of food will be determined through behavioural observations of feeding events known as 'trophallaxis' from videos of each of the colonies under the two scenarios. While this project is largely exploratory, as this aspect of ant behaviour has not been studied in great detail before, there are four main objectives: to determine and compare the rates of feeding under the two treatments; to explore the influence of or on the spatial structure of the colony members during food transmission; to explore

the amounts of food transmitted to individuals; and to explore the pathways used to distribute the food among members inside the nest. These objectives are set out in more detail in section 1.6 and stem from what we previously know about the behaviour of ants and other eusocial insects.

This introductory chapter is organised into the following sections: Eusociality; Immunity in eusocial insects; Resource distribution in eusocial insects; and the features of *T. albipennis*. In section 1.2 I give a definition of eusociality and outline the key components of colony organisation identified by Naug and Camazine as colony demography, the division of labour and the interaction network [2]. Division of labour is the existence of different types of individuals within a colony that perform different tasks whilst colony demography concerns the ratios of types of individuals in a colony and the life history schedule. The interaction network is crucial to the functioning of a colony particularly for resource distribution and reducing the spread of pathogens and parasites. Wilson [3], and Oster and Wilson [4], describe a eusocial insect colony as a 'factory within a fortress'. The factory component of this analogy is based on the efficiency of production of new generations inside the nest which is reliant on the provisioning of food to queen, brood and workers. The fortress component refers to the protective measures taken to defend a colony not only from predators but invasive species including pathogens and parasites. Immunity in eusocial insects is relevant to this study as the pathways used to transmit food could potentially create a route for a parasite or pathogen to spread through a colony. I therefore include a summary of studies on immunity (section 1.3) and feeding (section 1.4) in eusocial insect colonies as these are topics which are of particular relevance to this project. In section 1.5 I describe the characteristics of the study species used in this thesis, *T. albipennis*, explaining why it is a model species for this study and others including the large body of work conducted using this species by members of the AntLab at the University of Bristol.

1.2 Eusociality

Sociality among animals exists along a spectrum ranging from solitary, through increasing levels of sociality up to truly social or eusocial species. Solitary animals have little interaction with other members of their species except for mating purposes. In the middle of the spectrum social species might exhibit one or more of

the following features: parental care to their young; living in permanent groups with overlapping generations; cooperative care of young; cooperative foraging or hunting; and social learning. The most extreme form of sociality, eusociality, as originally defined by Edward Wilson [3], is phylogenetically rare having evolved independently within the Hymenoptera only 9 times [5]. Eusocial insects include members of the order Hymenoptera, that is all ant species, some bees and wasps, and the Isoptera, the termites. Outside the orders Hymenoptera and Isoptera eusociality only occurs in some aphids, thrips and crustaceans, a few other arthropods and two mammalian species, the naked mole rat and the Damaraland mole rat [6].

Eusociality is defined by three criteria: the adults care cooperatively for the young, e.g. see [7]; two or more generations of adults live in the same nest; and the members of each colony are divided into a reproductive 'royal' caste and a nonreproductive 'worker' caste (p.3 in [4]). Eusocial insect colonies normally have one or a relatively small number of reproductive individuals. Typically these are physiologically specialised 'queens' but in some cases where a specialised caste has not evolved (or has been lost) reproduction is undertaken by one or a few dominant females in the colony, e.g. [8]. Meanwhile the workers are either physiologically sterile or inhibited from reproduction by the dominant female or queen.

Eusociality has three main features which are relevant to this study. Firstly, colony demography which concerns the life cycle of a colony and the ratios of the different workers. Colony life cycles vary between species but the principal structure comprises: a founding stage where the virgin queen mates and founds a new nest; an ergonomic or growth stage during which workers are reared; and finally, when an optimal colony size is reached, a reproductive stage producing virgin queens and males (see Ch.3 in [9]). The four colonies used in this project are thought to be in the ergonomic phase. Mating strategies can affect the relatedness within a colony which in turn can affect the level of cooperation. There are two strategies commonly used: female calling syndrome and male aggregation syndrome [9]. In the first case, the virgin queen is mated only once, seen in *Leptothorax* species and the species used in this project *T. albipennis* [10], and generally results in high relatedness between offspring. In male aggregation the female mates multiple times, known as polyandry, as seen in desert ants and higher leaf cutting ants [11]. Colony demography also concerns the size and density of the colony. In some aspects larger colonies may benefit from an economy of

scale, for example reduced cost of maintenance [12]. These two parameters, size and density, will affect the frequency and contact structure of interactions among individuals [2] and therefore are likely to influence the distribution of food. The colonies used in this project have different colony sizes (and therefore densities as they are in the same sized nest) allowing us to explore to some extent the effect of density on food distribution.

The ecological hegemony of eusocial insects can be attributed to division of labour inside the nest and task specialisation [13]. It is this which makes eusocial insects more efficient over solitary insects as opposed to exploiting resources that solitary insects do not, (p.400 in [14]). For example, the division of labour in eusocial insects permits an extended period of parental care for young which could not be provided by solitary insects as often a single adult lifetime is too short [15]. The benefits of division of labour are described as: skill aquisition or increase of dexterity in each workman; spatial efficiency gained from not having to move from one task to another; and mechanical specialization to facilitate labour [16] (and see p.401 in [14]). Task specificity in individuals improves efficiency through improved handling of tasks [13]. Spencer et al. proposed that it is advantageous to maintain a specialization when the task is available and being able to switch when the environment changes [13]. As a result, the least specialised workers are in the majority and will specialise behaviourally according to the needs of the colony. In this project the colony requirements are likely to be for foragers and distributors within the nest. We will look to see if there is a shift towards these tasks during famine relief.

The sterile or effectively sterile workers in a colony can be subdivided into labour specialists or classes based on differences in probabilities of performing different tasks [17]. Tasks can be situated inside the nest such as brood care or outside such as foraging for food [18]. While it could be possible that each worker performs all tasks it has been observed that individuals show long term preferences for particular tasks [19]. A number of studies have shown that the division of labour in eusocial insects can be determined or influenced by a variety of factors including: physiology and morphology (p.237 in [20]); colony requirements [21]; the temperature larvae and pupae are raised at [22]; availability of work [19]; worker age [17]; worker nutrient status [23]; food provisioning to the brood [7]; and genetic diversity [11]. In the context of the current study the division of labour has important implications. The separation of workers into different

task groups may form a basis for how the distribution of food is organised both spatially and in terms of the transmission pathways.

A high proportion of the workers in a colony are inactive at any one time [24], workers are triggered to become active and perform a task when the stimulus for the task exceeds their response threshold. As a result of this we might expect very little activity during the control treatment when hunger thresholds are unlikely to be exceeded very often. Individual response thresholds to tasks in ants, honeybees and bumblebees are determined by internal factors such as genetic predisposition, physiology, physical caste, age or individual experience, e.g. [25]. As a consequence of different individual response thresholds we may expect there to be differences in individual feeding behaviour, particularly apparent under the famine relief treatment. Response thresholds can also be influenced by external factors such as demand for food or distance from nest to food patches [26].

In general colony functions such as nest maintenance, foraging and reproduction are coordinated without any central control or environmental template and form complex spatio-temporal patterns (p.403 in [14]). This feature is known as self organization whereby a global pattern formation results from interactions internal to the system without intervention from external directing influences (p.7-8 in [27]). These patterns and therefore cohesion and coordination of colony functions are reliant on a high frequency of social interactions between colony members.

The purpose of social interactions is to transfer resources and information vital in decision making among colony members, e.g. see [28, 29]. Social interactions also serve to improve the hygiene of the colony, for example, in the case of mutual grooming known as allogrooming [30]. Hygiene is a high priority in colonies which are particularly vulnerable to exploitation by pathogens and parasites, see section 1.3. Social contacts also serve to spread the colony odour used to recognise nest mates from intruders which typically elicit an aggressive response (Ch.5 in [9]). Colony odour is made up of cuticular hydrocarbons detected by chemoreception during brief antennal contacts. Work by Wagner et. al showed that in harvester ants the hydrocarbon profile of outside workers is altered under warm dry conditions [31]. This change in profile is used by workers inside the nest to detect external workers [32]. There is also some evidence that workers are capable of recognising individuals as well as classes of workers, for example see [33]. The existence of the ability to recognise individuals or classes of individuals within a colony is important to this study if the workers have a preference for which individuals they transmit food to or receive from.

The data collected during this study can be used to deduce the pathways of food transmission among individuals in a colony. These transmission pathways could potentially be analysed using techniques from social network analysis. The use of social networks to analyse animal groups is a rapidly growing theme in biology. In vertebrate studies social networks are generally constructed from observing patterns of social interactions or simply proximity between individuals, e.g. [34, 35], and then might be used to infer how a process such as a disease would spread over the network, e.g. [36]. The relatively fewer studies that use social networks in the invertebrates tend to construct a network of an actual instance of a process, then aim to deduce the pattern of social structure from that instance, see [37, 38] for example. The difference between these two approaches means that many of the measures devised to analyse networks of the first type, i.e. of pattern, are not appropriate for the analysis of networks constructed from a process. As an example, a measure often used is the 'clustering coefficient', (see e.g. [39, 34]), which gives the probability that if individual A is connected to B and to C, then B and C are connected (i.e. the existence of triangles in a network) [40]. In a directed network with causality (time ordered events) this measure is unlikely to be informative. Therefore throughout this thesis, the transmission pathways deduced may be referred to as 'networks' but they cannot be approached in the same way as social networks constructed by observing and averaging social interactions.

The following two sections address two of the main functions of social interactions: how eusocial insects combat pathogens and parasites; and the distribution of resources, primarily food, within the colony. The aim of this project is to explore how eusocial insects distribute food to their members after a period of famine. It is vital to know what has already been found in this area from previous studies on feeding behaviour and food distribution. Eusocial insect colonies are particularly prone to infection from pathogens and therefore we might expect them to take some precautions during resource distribution. It is therefore useful to know what approaches and strategies eusocial insects use to combat pathogens.

1.3 Immunity in eusocial insects

In eusocial insects and indeed any social animal there are several factors that increase the risk of becoming infected by parasites and pathogens. These include:

living at high density with frequent contacts [41, 2]; low genetic diversity [42]; and having a fixed nest site [30]. It has been shown that allogrooming, a hygienic behaviour, could actually increase the pathogen transmission rates under conditions of periodic exposure [30]. Similarly, it has been shown that several pathogens are spread during trophallaxis among workers such as chronic bee paralysis virus [43] and some rhabditid nematodes in ants [41]. Meanwhile simply being in close proximity to nest-mates can also spread pathogens such as the fungus *Alternaria tennuis* [44] and the mite *Acarapis woodi* in bees [41]. Colony members of eusocial insects are typically closely related, particularly in monogynous species [45]. As a result of the low genetic variation between workers the level of immunity in a colony to a certain pathogen will be low [42]. Increased genetic variation in colonies through polyandry has been shown to improve a colony's resistance to disease [46].

Eusocial insects have evolved several strategies to counter or prevent infections by parasites and pathogens. Resistance against parasites in a eusocial insect colony, similar to that within a single multicellular organism, is comprised of three levels: *border defences*, *soma defences* and *germ line defences* [47]. The border defences act to prevent the initial uptake of a parasite, i.e. entry to the colony, soma defences act to prevent the spread among workers and germ line defences act to prevent the parasite spreading to the reproductive individuals i.e. the queen and males. These defences include: parasite avoidance [48]; auto- and allo-grooming; antibiotic glandular secretions [49]; removal of dead or diseased individuals from the nest [50]; collecting plant materials rich in volatile compounds [51]; and waste management [52].

An important question relevant to this project is whether workers can detect a harmful substance in food introduced to the colony. Many parasites are contracted through feeding on contaminated food, for example the fungus *Beauveria bassiana* in fire ants [53] and bacterial infections [54]. A study on bumblebees, *Bombus terrestris* (L.), found that the foragers drinking solutions laced with harmful substances could not discriminate between harmful and safe food with a negative effect on the number of offspring as a result [55]. This suggests that foragers are not always able to determine whether the food they have collected is safe or not. If this is true in general, we might expect that the behaviour of colony members inside the nest during food distribution may be adapted to counter the negative effects of potentially harmful incoming food, for example by not distributing to brood carers.

In some cases exposure to pathogens can have long-term positive consequences for the colony. Specific immune priming is the lasting specific protection after an initial pathogen exposure [56]. This has been demonstrated in *Bombus terrestris* with increased protection upon secondary exposure of bacterial pathogens [56]. This protection is increased by social transfer of infection resistance, shown in termites [57]. Individuals improved their ability to resist infection from a fungus when they were mixed with and made contact with previously immunized nestmates. This social transfer has also been identified in garden ants, *Lasius neglectus*, exposed to a fungal parasite [58]. Individuals which were exposed to nestmates treated with a sham-control or non-treated nestmates were 1.5 and 1.7 times more susceptible to infection than individuals which had interaction with nestmates treated with the actual live spores of the *Metarhizium* parasite. The study also showed that ants treated with the live spores stayed away from the brood chamber and the queen, probably because they represent the future investment of the colony, an example of a germ line defence.

These studies raise the question: if a parasite succeeds in breaking the border defenses and entering the colony, is it more beneficial to spread it around the colony to facilitate social transfer of infection resistance or would it be more advantageous to contain the parasite in the smallest number of workers as possible, thereby partitioning the colony? The answer will depend on the type of parasite or pathogen the colony has been exposed to and colony demography. There may be clues in the interaction networks found in this and other studies as to whether colonies try to spread or contain a pathogen under certain conditions.

A handful of recent papers have used the technique of tracking individuals and behavioural observations to build an interaction network through which a parasite or pathogen might spread, see [38, 37, 59, 2]. These studies tend to use either small colony sizes or a subset of the interactions. Colonies of 5 to 7 bumblebees were used to show that the spread of a contagious pathogen *Crithidia bombi* in a colony is determined by contact network characteristics of the host [38]. Another study tracked the first and second order events of individual honeybees in a colony of 1000 to create food transmission networks under two treatments representing short and long infectious periods [37]. The study showed that the spatial organisation of the colony influenced the network structure providing some immunity to younger individuals and a high resilience to the removal of individuals. A study in the social wasp *Ropalidia marginata*, using colonies of 8 to 40 individuals, revealed that heterogeneity in connectivity increases as colony size increases,

with a few individuals in larger colonies being responsible for a disproportionate amount of interactions [59]. Naug and Camazine developed a simple model to see how the three features of colony organisation might influence the transmission of pathogens [2]. Their model showed that while division of labour alone did not reduce the spread, in combination with a heterogenous contact network the spread of a pathogen was likely to be reduced. The contact network represents contacts made during allogrooming and trophallaxis. From these studies we might make several predictions about the structure of the transmission networks in this study: they might be influenced by the spatial organisation; they are likely to be structured to protect the young; and more heterogeneous contact structures are likely in larger colonies. Given that the contact structure influences the spread of the pathogen we may see some measures taken to prevent or even maximise spread of pathogens with the food. If larger colonies have a more heterogeneous contact structure and this is known to reduce the spread of pathogens, they might be better at preventing the spread, however the effects of heterogeneity in the contact structure may only be enough to off-set the effect of living at a higher density.

As mentioned previously, in this study while not directly observing the spread of a parasite or pathogen the pathways formed during the transmission of food may provide an indication of how one might spread and how the colony might manage this risk. The following section addresses the features of resource distribution in eusocial insects (inside and outside the nest) which are relevant to this study.

1.4 Resource provision and distribution in eusocial insect colonies

The aim of this project is to explore resource distribution in eusocial insect colonies, in particular the distribution of liquid food among workers inside the nest. Provision of food to a colony can be broken down into several inter-linked components: assessing hunger and initiating foragers to leave the nest; searching for food; laying down trails when food is located; recruiting additional foragers when necessary; transporting food back to the nest; distributing the food within the nest to the appropriate individuals and contingency plans for emergencies. It is clear that many, if not all, of these tasks rely on interactions between individuals within and between different task groups.

Different food types are required and utilized by different members of the colony. In general sugars are used by the adult workers, lipids by the workers and some larvae, while protein is used by the larvae and egg laying queens [60]. Liquid food is transferred between individuals via trophallaxis while other types of food are carried into the nest whole such as *Drosophila* flies, a source of protein for larvae, and in some species trophic eggs are formed and fed mainly to queens and larvae (p.103 in [61]). In this project the focus is on the distribution of liquid sugar which is primarily utilized by workers. Therefore provisioning to the brood, queen and males is not going to be investigated however several studies have focused on this aspect of feeding, for example see [62, 63, 64]. Larvae are generally fed by an older generation of adult workers; work on fire ants showed that the larvae are fed in tiny increments over hundreds of interactions with many workers over several hours thus creating a uniform distribution of food among the larvae [64]. In this project we will look to see if a similar approach is used to feed workers or if they receive large amounts of food in one interaction.

Previous studies have shown that foragers are able to assess the hunger of the colony and the quality of the food they collect, e.g. see [60] and [65]. Work by Sorensen et al. [60] on fire ants, *Solenopsis invicta*, has shown that foragers assess the hunger of the colony via a class of workers called 'reserves' who relay food between the nurses and foragers. In honey bees foragers have been shown to assess the colony hunger level via pollen storage levels which, if found to be low, cause pollen intake rates and therefore foraging effort to increase [65]. The increase in effort was largely attributable to individual changes in foraging behaviour, e.g. increasing pollen load size, as opposed to an increase in the overall proportion of foragers. When demand for food is low, foragers are known to assess the quality of the food they find, for example see [66, 67]. In honey bees under high levels of pollen storage inside the nest, foraging trips were longer in duration than under low pollen storage [65]. It is thought that this is because when there is less demand for it the bees are under less pressure to collect pollen so they have more time to assess the quality. In contrast when supplies are running low and demand is high the priority is the rapid influx of pollen regardless of quality. Foragers also spent less time in the hive between trips under low pollen storage as many receiver bees were available to unload the pollen from them quickly demonstrating how the receivers regulate the feedback loop between the foragers and the pollen storage. These findings have important implications for this study: will the ants respond to the increased hunger of the colony by increasing the proportion of foragers or will individual foragers increase their own work effort? It is also important to ask

why the foragers choose a certain strategy, it may be that if the food source is close to the nest entrance it is more efficient to increase individual effort instead of recruiting more individuals.

Once the food has been located the foragers must transport it efficiently back to nest, speed is important when the nutritional state of the colony is low, when there are competing colonies that might also deplete the food source and when there are predators that might prey on the foragers. One way to speed up the transfer of food back to the nest is to recruit more foragers. Existing foragers recruit new foragers by various means for example using a 'waggle dance' in honey bees [68]; tandem running in ants [9]; and through pheromone trails deposited by foragers or scouts [69]. von Frisch suggested that in honey bees during intervals between dancing foragers would donate a small amount of nectar to potential recruits, [70], a behaviour we can look for in this study. The purpose is likely to be to transfer information about the presence and quality of the food. Recruiting more foragers is beneficial because it can make the transportation of food back to the nest more efficient. The disadvantage is that a larger proportion of the colony will be undertaking this risky task. Mortality rates among foragers are high and foraging is often undertaken by workers with a shorter life expectancy [71].

Inside the nest liquid food is transferred from foragers to other colony members in almost all species of ant and many other eusocial insects, by a process called 'trophallaxis'. During trophallaxis between two adults, the forager is induced to regurgitate food from her crop by a nest-mate touching her fore-legs or antennae to the foragers labium (lower mouth plate) and tapping repeatedly (p.258 in [9]). Once trophallaxis has been initiated the food then passes into the mouth and to the crop of the receiver. It is this interaction between workers that forms the basis of the transmission pathways analysed in this thesis. It is thought that cues other than the antennal tapping of a soliciting nestmate may also be used to initiate trophallaxis such as pheromones, the smell of the food and head volatiles [72]. It is important to note that trophallaxis does not only occur with foragers as the donors, indeed non-foraging workers donate food to each other, e.g. [73], to larvae, e.g. [64], to the queen, e.g. [74], drones and in honeybees at least, to foragers before starting a foraging flight [72]. The crops of all the members of the colony make up the 'social stomach', a reserve store for the entire colony (p.103 in [61]). Trophallaxis is much more important to colony life than simply a means of transferring food between two individuals; important information is also shared

about the hunger state of the recipient and the quality and availability of food from the donor. At the same time pheromones, [75], and gut symbionts, [76], are shared.

1.4.1 Resource distribution within the nest in eusocial insects

Several studies have looked at resource distribution among workers inside the nest in ants [73, 77, 78, 79], bees [80, 81, 72, 82] and termites [83, 84]. These studies have revealed several interesting features so far. Firstly the variation in the time taken for food to reach all members of the worker population across different species which ranged from 30 minutes [78] to 3 days [83]. It was noted that such rapid transmission was surprising during periods when food was abundant and therefore not likely to be in high demand [77]. If transmission is rapid through the worker population, this has implication for how a pathogen or insecticide may also spread [83]. One study showed an exponential recovery fit to the increase in the number of fed ants as a function of time time,

$$\frac{\mathrm{d}q(t)}{\mathrm{d}t} = \alpha(1 - q(t)) \tag{1.1}$$

where $q(t)$ is the quantity harvested as a fraction of the 'colony desired harvested volume', K [78]. The authors conclude that the flow of food into the colony is proportional to the number of foragers and the colony desired harvested volume is less than the potential storage capacity of the colony. Perhaps it is not beneficial for the colony to reach complete saturation if some workers are required to remain available to feed the brood which uses a different food type. We will look in this study to see if the potential capacity of the colony is reached. One study observed that workers received food in trophallaxis in groups of up to eight recipients to one donor after a period of starvation [79]. This is likely to be a mechanism to feed workers quickly when new food is found.

Secondly several studies suggested that chains of transmission were used to distribute food among the worker population, see [73, 77, 83], but could not verify this due to the technique applied (i.e. following the progress of labeled food). In this study we will determine the pathways used to distribute food among workers and investigate whether there is any structure to these chains. Such chains

of transmission are thought to optimize colony homeostasis particularly when transfer of gut symbiotes or pheromones is involved [83].

Another finding of these studies was that the queen and larvae tended to receive very little food or received it much later than the workers, [73, 77]. Given that the queen and larvae tend to utilize a different food type to the workers, [60], this result is perhaps not surprising. In addition, the delay in feeding them (whether intentional or constrained by the spatial organisation inside the nest) could be a method for protecting these important colony members against pathogens which may be unintentionally spread with the food. Eisner and Wilson's study inferred that the gut content of the colonies tended to uniformity [73] in contrast to other studies which found an uneven distribution of food among workers, [80, 78], with nurse bees receiving less food than other classes [80]. Meanwhile a study in fire ants found that in smaller colonies small individuals received most frequently while the converse was true in larger colonies [79]. One of the aspects this study will look at is whether workers receive equal amounts of food and if not which individuals are receiving large amounts. Crailsheim deduced that honey bees feed other bees of a similar age and function due to the organization inside the nest [72]. Similarly, Free found that honeybees donated to workers of all ages but preferentially to those of a similar age to themselves [82]. In this study we will look to see if there is a preference to feed workers from certain task groups.

A further finding in one study showed, by spatially monitoring the centre of gravity of food, that the food became more concentrated as a function time and the centre stabilised after thirty minutes [78]. These spatial results indicate that food was heterogeneously distributed within the nest. In this study I will look at the final spatial distribution of food inside the nest to determine whether it is concentrated in one area.

1.4.2 Starvation periods

Eusocial insect colonies often live in changing and unpredictable environments. There may be periods a colony will have to endure without a regular food source; particularly in the winter ant colonies must survive lengthy periods of low food availability. Colonies have evolved strategies that enable them to deal with such times including: increasing or decreasing the amount of scouting, e.g. [79] and [85]; and eating eggs and larvae [86, 72]. Experimentally colonies have been

shown to survive relatively long periods of starvation, for example a study on the ant *Camponotus mus* deprived colonies for 15 days [87], similarly colonies of *T.albipennis* were starved for two weeks with only access to water [24]. A study on *Temnothorax rugatulus* showed that colonies survived eight months of starvation [85]. During this time brood decline started earliest followed by workers and queen decline started latest. The study found that rates of trophallaxis increased during the starvation period and activity bursts were more frequent but shorter leading to an overall decrease in the level of activity. A study on *Leptothorax acervorum* which starved colonies for between eight and sixteen days showed that ants tended to remain inactive unless triggered by an active ant [88]. The starvation period used in this study is 48 hours which is a relatively short time in comparison to some studies but represents a period in the wild when foragers are unable to forage (for example due to heavy rain) or no food is available. Therefore the ants will not be completely starved, so we don't expect eggs or larvae to be eaten, but will be hungry when the food is re-introduced. In this project the aim is to look at how the ant colonies distribute the food after a period of starvation, however there may be clues in the workers' behaviour as to how they cope during longer periods of food shortage. The following section describes the salient features of the study species *T. albipennis*.

1.5 *Temnothorax albipennis*

T.albipennis is distributed within Europe, with some coastal sites in the UK as the northern edge of its range. For this study we use *T.albipennis* colonies from the coastal area of Portland in Dorset, Britain. There are several features of the ant species *Temnothorax albipennis*[1] which make it an ideal species for studies in socio-biology such as this one. Firstly the relatively small colony size, ranging from around fifty to two hundred monomorphic workers [10], means an entire colony is a manageable unit to work with and study at the individual level. In comparison, other ant species that are studied in the laboratory, for example the fire ant *Solenopsis invicta* [64, 60], are typically much larger and it would not be feasible to study the whole colony at the individual level.

[1]The species *Temnothorax albipennis* was originally misidentified in Great Britain as *Leptothorax tuberum* Fabricius until it was re-identified as *Leptothorax albipennis* in 1998 [89], and finally renamed *Temnothorax albipennis* in 2003 [90].

T.albipennis founds new colonies during the summer by colony fission whereby the colony splits and the virgin queen typically mates with one male, i.e. is monogynous [91]. The species shows seasonal polydomy where a colony splits during the warmer months (May to August) and then reunites in the autumn [10]. It is thought that the increase in colony activity and brood size in the warmer temperatures forces the colony to seek further nest space resulting in the observed seasonal polydomy. The colonies used in this study were collected at the end of September after colony fragments would have re-united thus ensuring that the maximum number of workers were collected and that each colony was queen-right.

In the wild colonies form their nests inside flat crevices in rocks with typically a single circular chamber with one entrance, see figure 1-1. The single nest entrance might make the nest easier to defend against invading species, e.g. slave makers, but could also be used as a tool for coordinating the foraging and feeding behaviour of the colony. The perimeter wall is the only part of the nest constructed by the colony and is built using small particles of debris and grit [92]. Studies in the laboratory showed that when initially creating the nest wall after an emigration building workers use the cluster of sheltering nest-mates inside the nest as a template and simple behavioural rules for their building activity [92]. As a result the area the workers allow for the internal cavity depends on the number of workers in the colony allowing on average around 5mm^2 per ant, (worker bodylength $\approx$ 2mm) [92].

When compared to the nests of other ant species, which can be complex and three dimensional with multiple chambers and tunnels, see for example [93, 94], the geometry of *T. albipennis* nests is approximately two dimensional. This means that an entire colony can be easily extracted from its natural nest by aspiration and re-housed in the laboratory between two glass microscope slides [95]. This design enables the observer to monitor the whole colony and to view and record every action that occurs inside the nest. The artificial nest is usually placed inside a larger Petri dish or arena that is coated in Fluon to prevent the ants escaping. This design makes a nest within a small 'world' for the colony. For this project no building materials were provided for the ants but nest walls were included by placing a layer of 1mm thick cardboard with a rectangle cut out the middle between the two microscope slides of the artificial nest. This means that the area of the internal cavity is greater than the 5mm^2 per ant. I will investigate

Figure 1-1: *Photograph of a* Temnothorax albipennis *nest in the wild. The rock crevice has been opened to reveal the nest cavity within.*

the actual area used, i.e. the effective density, in each colony and whether famine relief has an effect on this.

The potential for *T.albipennis* as a model eusocial species for behavioural ecology was recognised by Professor Nigel Franks, the head of the Ant Laboratory at the University of Bristol. The Ant Laboratory group has used variations on this experimental system to make a significant contribution to the knowledge we have concerning *T.albipennis* and other ant species. Their large body of work includes the discovery of spatial fidelity zones in *T.albipennis* [95]; brood sorting [96, 7]; self-organization in nest construction [92]; the phenomenon of 'Move to improve' in colony emigration [97]; the preferable features when choosing a new nest [98, 48, 99]; the use of quorum sensing during colony emigration [100, 101, 102]; the use of 'Buffon's needle' algorithm to estimate the area of a potential nest [103]; how the queen is protected during transport in emigration [104]; and recently the technique of tandem running where one ant leads another ant from the nest to a target, e.g. a food source, as the first example of teaching with feedback in non-humans [105, 106].

The existence of a strong spatial structure inside the nest of *Temnothorax* colonies, [107, 95, 108], is an important feature for this project. The studies, which initially used *T. unifasciatus* a closely related species to *T. albipennis*, showed that

the structure is based on 'spatial fidelity zones'. Firstly 'stations' inside the nest where workers could be found were identified [107]. These were: deepest in the nest for nurses of eggs and microlarvae; between the first station and the middle of nest for nurses of older larvae; between the middle of nest and the nest entrance for generalists and finally in the vicinity of the nest entrance for foragers and exit guards. In the second paper each individual worker was found to have a movement zone of limited area [95]. From a series of photographs each worker's position inside the nest was recorded. Each worker's space use was then reduced to one dimension, the distance from the centre of the brood as this is the biological centre of the colony. The inter-quartile range of each worker's distance from the centre was calculated for workers that had at least 5 recorded positions inside the nest (a maximum of 100 positions per worker). A linear relationship between the upper and lower quartiles was revealed demonstrating that the workers were using zones in the form of concentric circles centered on the brood expanding out to the periphery of the nest with partial overlap. The division of labour was found to be flexibly organised along this continuum of spatial fidelity zones as opposed to being strictly based on worker age. In an accompanying paper Tofts outlines an algorithm known as 'foraging for work' for task allocation based on the availability of work [19]. In this algorithm, if an individual fails to find work within its current task for some period of time it may then change task in order to attempt to find work. As a result the correct number of workers are allocated to a task in proportion to the relative amount of work currently available in that task and assumes a linear succession of tasks. Tofts shows that such a mechanism will result in an aged-based structure which is weakly seen in the empirical study [107]. Several other studies have also focused on task allocation and division of labour in *T. albipennis*, specifically looking at the roles of corpulence [23, 109, 110], worker age [110], previous activity [109, 110] and colony size [24] on organisation of work. These studies showed that task allocation was only weakly associated with age, colony size did not influence individual specialisation, however nutrient status, particularly lipid stores, influences role predisposition.

A later paper using *T. albipennis* confirms the existence of spatial fidelity zones in this species and showed that after enforced emigration workers re-adopted their previous spatial positions inside the new nest [108]. This phenomenon is known as 'social resilience' and is maintained even when the queen, brood and large proportion of workers are removed during the emigration. It means that the colony organisation is robust to drastic changes that are expected to occur in this species due to its ecology, see [10]. The existence of a spatial structure inside

the nest is of particular importance to this study as potentially it could influence the process of food distribution. The spatial structure implies that inside the nest the workers are not 'well mixed', i.e. the workers are limited in which other workers they are likely to have contact with. This is likely to influence who feeds whom in the pathways used for food distribution and we will look to see if this structure is upheld during famine relief.

The spatial structure of *T. albipennis* is centered on the brood pile. Brood items can be categorized into large larvae, medium larvae, microlarvae and eggs. The workers of *Temnothorax* colonies actively arrange the brood items inside the nest [7]; microlarvae and eggs are usually arranged into a small and homogeneous group which can be considered the biological centre of the colony [95]. The larger larvae are placed in progressively larger rings around the central cluster and pupae in an intermediate ring between medium and large larvae. Each brood item has a 'Domain of care' around it, an area within which points are closer to that item than any other so a worker within that area will tend to that brood item, [7]. The brood pile in the rectangular artificial nests used in this project can be arranged into concentric rings, semi-rings or straight bands [95]. The spatial organization of the brood pile may influence the movements of the workers inside the nest, for example as pointed out in [95] a location with a large larvae probably cannot be occupied by a worker and therefore may influence workers movement during food transmission.

T.albipennis subsists almost entirely on scavenged material, for example dead insects, bird droppings, animal waste, fruit fragments and vertebrate carcasses [111]. This means that the value of food found by foragers and its spatial location is unpredictable. Species which rely on scavenging for food are therefore expected to develop mechanisms for a rapid concentration of nest-mates at a food item to prevent losing food through competition from other colonies [111]. In this study we will compare the number of ants that leave the nest to see if more workers are recruited when a new food source is provided and why a large recruitment effort is not always the best solution. The larvae can be fed with fragments of insects carried whole into the nest, under laboratory conditions *Drosophila* flies are used.

Given the vast amount of research, it is perhaps surprising that so little is known about the feeding behaviour and the distribution of food inside the nest among workers of *T.albipennis* and many other ant species. The outcomes of this study will make original contributions to this area.

1.6 Thesis objectives and overview

This thesis explores how four colonies of *Temnothorax albipennis* distribute food after a period of starvation in comparison to under normal (food abundant) conditions. Guided by the knowledge gained from previous studies outlined in this chapter, I have devised four main objectives which will be addressed in the 3 main results chapters (4, 5 and 6):

A. To determine and compare the rate of feeding under the two treatments and determine how faster rates are achieved. It is reasonable to predict that feeding will occur at a higher rate under the famine relief treatment compared to during the control. We will explore how the colonies achieve achieve faster feeding. From the literature we might expect to observe several features such as feeding in groups [79]; a higher rate of activity of workers [88]; and possibly an economy of scale in large colonies (which may also be present under normal conditions) [12].

B. To explore the spatial structure of the workers during the two treatments and determine whether it is maintained or abandoned during famine relief. The study species has a strong spatial structure centered on the brood expanding out in concentric rings. If this structure is maintained during famine relief it is likely to influence the organisation of feeding. If abandoned the roles of the workers may still be important so I will classify the workers based on their space use.

C. To determine the amounts of food distributed to individuals, establish whether the food is distributed evenly among workers and whether the colony capacity is reached. Previous studies have shown that crop contents tend to uniformity [73], while others have shown an uneven distribution of food among the workers [80, 78]. The amount an individual receives may be related to their role, for example some studies have shown brood carers to receive the least amount of food [80]. It has also been shown that colonies do not reach their potential storage capacity [78]. I will use classes based on individual space use (see objective B) to determine which task group receives the most amount of food.

D. To deduce the structure of transmission pathways used to distribute food among workers and determine if there are preferences

for who feeds whom. Several studies in other species have inferred chains of transmission among colony members, e.g. [73]. We might expect these chains to be based on the spatial structure of the workers. We might expect feeding to be in partitioned chains of transmission (based on spatial structure) to minimize spread of harmful substances. Alternatively, feeding (and exposure to foragers) may mixed to promote the spread of social immunity.

The remainder of this thesis is set out as follows. In Chapter 2 I describe the materials and methods for the experiment including: experimental design; details of the two treatments; the tracking and data collection processes; the process used to verify the trophallaxis data; and the data structure before refinements made in Chapter 6 were implemented.

In Chapter 3 I present several of the general features and responses to famine relief that were apparent from an early stage and thus influenced the direction of investigations in this project. These features include the variation in demographic and geometric properties between the four colonies used; the act of feeding multiple recipients simultaneously from one donor in 'rosettes' and the level of brood coverage throughout the famine relief treatment; and feeding interactions involving the queen.

Chapters 4, 5 and 6 present the main results addressing the four objectives set out above. Objective A is primarily addressed in Chapter 4 which presents detailed results from a temporal perspective. This chapter explores the levels of feeding activity as a function of time, how quickly the colonies relieve the famine, the pathways used to relieve the famine and the use of the previously mentioned 'rosettes' to facilitate food distribution. I show that under the famine relief treatment all four colonies are much more efficient at distributing the food compared to under the control and the increase in the number of fed ants is fit by a recovery exponential as seen in [78]. The model consistent with their behaviour assumes a well mixed system which is contrary to what we know about the spatial structure of this species.

In Chapter 5 I explore the space use of the colonies at a colony level, group level and individual level. I test whether there is a difference in space use between the two treatments in terms of where the ants are and the area they cover (Objective B). I also look at the number of trips outside the nest external ants make and show that there is an increase in individual forager effort during famine relief. I show

how the individuals adapt their space use to facilitate efficient food distribution by abandoning their spatial fidelity zones and increasing individual area of space use during famine relief (Objective A). I go on to categorise the ants based on which area of the nest they used most during the control treatment and their behaviour in the two treatments. These categories are used in Chapter 6 to look at various aspects of the food distribution.

In Chapter 6, using the durations of the trophallaxis events to estimate the amounts of food transmitted I investigate the amounts of food distributed and the transmission pathways (Objectives C and D). I show an un-even distribution of net-food among workers and that an individual's role influences how much food they receive. The careful analysis of the data reveals that a small number of ants in each colony have stored food before the famine relief treatment which they subsequently donate to nest-mates in parallel with the new food provided by foragers. Given that ant colonies are vulnerable to pathogens which could be spread via trophallaxis I also investigate how the networks of interactions may have been formed in a way that reduces the risk of harmful substances spreading through the colony. I find that to an extent the colonies are undertaking some risk management with preliminary evidence that the transmission networks are partitioned.

In Chapter 7 I summarise the main findings of this project which are consistent across all four colonies and their relevance to the field. I describe how this project could be extended and improved to broaden our knowledge of resource distribution in eusocial insect colonies.

Chapter 2

Materials and methods

In this chapter the methods used during the focal experiment of this project are described, followed by an explanation of the data collection process. Due to the difficulty in the data collection process and complexity of the resulting data set I designed and carried out a verification procedure to increase the accuracy of the collected data. This stage is detailed later in this chapter. Finally I lay out the resulting structure of the data that was obtained from this set of experiments.

Parts of the experiment described in this chapter were carried out by the people as listed below, without whom this study would not have been possible: the colonies of ants were collected by Nigel Franks, Ana Sendova-Franks, Richard James and Tom Richardson; the experiments, marking of the ants and initial trophallaxis data collection were done by students Benjamin Wulf and Thomas Klimek of the University of Applied Sciences Bremen, supervised by Ana Sendova-Franks and Nigel Franks. My involvement commences at the data collection (spatial data), verification and analysis stages.

2.1 Experimental design

Eight complete, queen-right *Temnothorax albipennis* colonies were collected by aspiration in Dorset, U.K. on the 30th September 2006. Colony sizes ranged from 42 to 95 individual ants (including one queen in each). At this time of year colony fragments re-unite for the winter and most workers are inside the nest making them easy to collect. To ensure as many workers as possible were collected the collectors waited outside the nests for an hour to catch returning foragers. During experimentation, the colonies were kept at a constant temperature of 24°C and natural light:dark regime in the ant laboratory of the University of Bristol. Each colony was housed between two microscope slides (45 × 36 mm, area 1620 mm^2), separated by an edge of cardboard (1mm thickness) with a gap (2 mm wide) in one edge creating a single entrance into the artificial nest, see figure 2-1. The total internal area of the nest cavity is approximately 1248 mm^2 (39 × 32 mm) which allows between 13 and 30 mm^2 per ant depending on colony size. This is larger than the 5 mm^2 per ant that we know this species creates when allowed to build their own nest wall, [92]. In this experiment no building materials were provided for the colonies therefore the differing colony sizes mean the ants are at different densities within the nests. The effect of density will be explored in Chapter 5 which focuses on the space use of the ants during resource distribution.

Figure 2-1 shows a colony inside an artificial nest as seen from above. The artificial nests were then placed inside Petri dishes (105 x 105 x 20 mm) the inside vertical walls of which were prepared with Fluon© to prevent the ants from climbing out of the dish, see figure 2-2. On the same day each week, under controlled conditions, each colony was provided with one tube of water, two droplets of honey solution (one part honey solution ten parts water) and three *Drosophila* flies. These resources were provided outside the nest but within the Petri dish. The two droplets of honey solution were an excess supply of food for the colonies and were never fully depleted for the duration they were present.

2.1.1 Marking the ants

To enable the identification of each individual every ant in all colonies was marked with a unique ID code with the exception of the queen who is recognisable by her larger size. Marking of *T.albipennis* is performed using paints markings on the

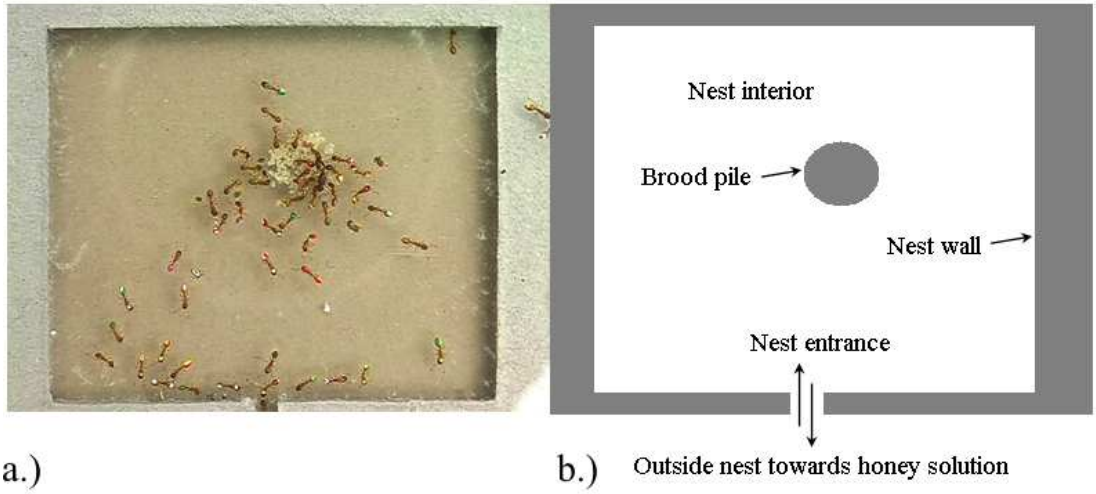

Figure 2-1: *a.) Photograph of an artificial nest as it would be filmed. Here you can make out the coloured paint markings on the ants that make it possible to identify each individual. b.) Schematic of an artificial nest detailing the brood, nest entrance and nest walls. The honey solution,* Drosophila *flies and water are provided outside the nest and are not in the frame of view of the cameras when filming. Width and height of internal cavity are 39 and 32 mm respectively.*

head, thorax and gaster [95]. This allows easy identification of individual ants without any mortality being attributed to the marking technique. The individual ant is anaesthetized with carbon dioxide before being placed in a slit in a small block of sponge [107]. Tiny droplets of polycarbonate, ketone soluble, model paint are applied to the ant's gaster using a very thin entomological pin set in a match stick and then the marked ant is placed in a new petri dish with the new nest in. If marked ants are returned to their old nest the unmarked nest-mates are likely to act aggressively towards them as the paint is an unfamiliar smell. Studies have shown that a completely alien element will illicit high aggression from nest-mates, for example see [112]. Once the whole colony has been marked and placed in the new nest the paint odour is uniform across the colony so illicits no aggressive response. The species used in [107] was *Temnothorax unifasciatus* a closely related species to *T.albipennis*, however there are several more recent studies in *T.albipennis* which use this well refined technique, for example [101, 24, 108]. The authors remark that after the colony was marked "the ants seem to groom one another more often than usual for the first few days after they have been marked" but this period is negligible in comparison to how long the paint marks last, which can be many months.

Figure 2-3 shows several marked ants drinking from the honey solution; their

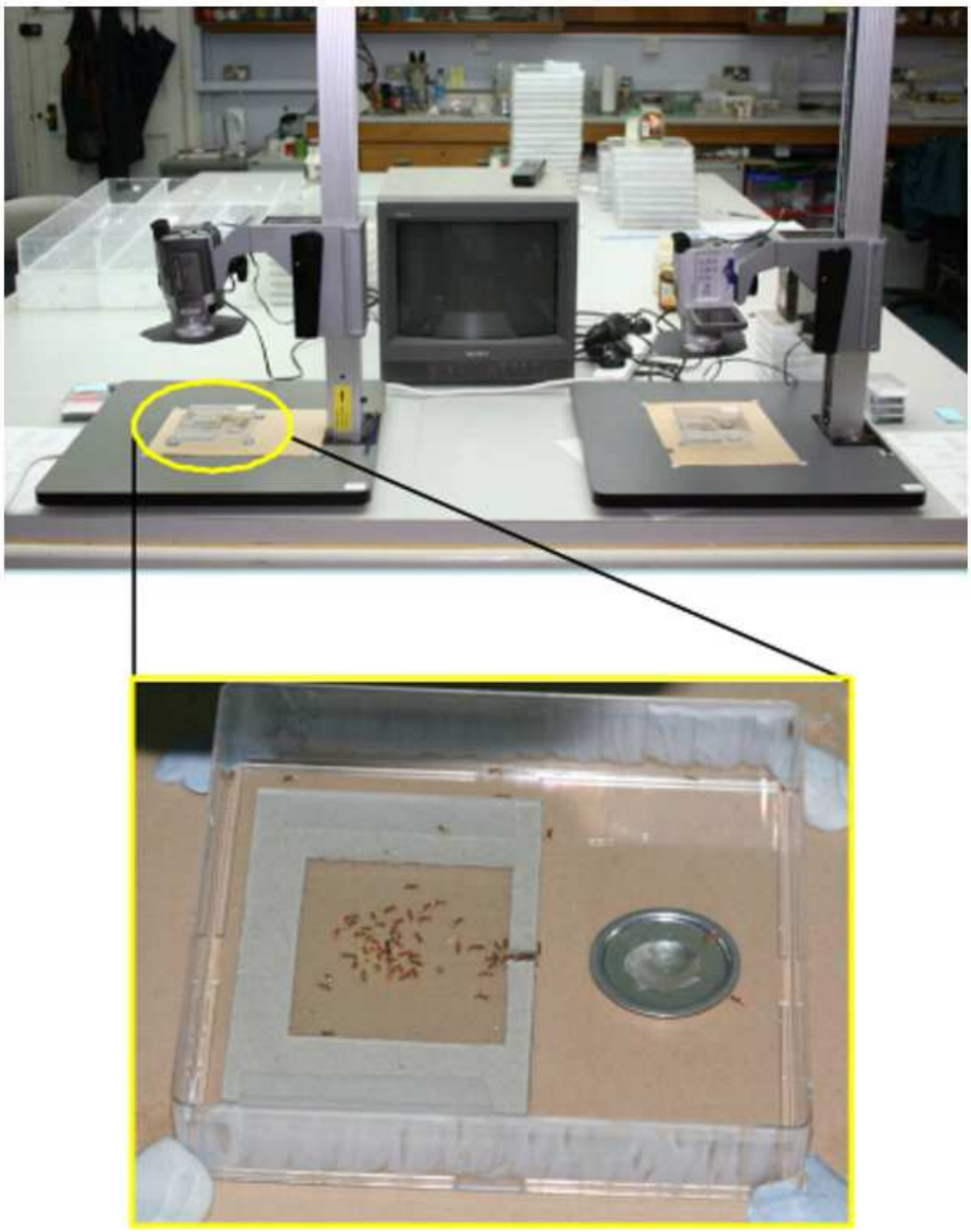

Figure 2-2: *Photograph of the experimental setup within the lab showing two colonies being filmed from above. Inset shows the arrangement within the Petri dish: the artificial nest to the left and the planchet containing the honey solution to the right.*

unique colour codes are visible. Each code consists of a unique permutation of colours on the head, H, thorax, T, and left side and right side of gaster, Gl and Gr, or a dot, D, on a main colour on the gaster, Gm.

Figure 2-3: *Photograph of several marked workers drinking from the honey solution to highlight the individual colour codes. As an example, if 'O' is orange and 'G' is green, the code for the second ant from the right would be: H=O,T=-,Gl=-,Gr=-,D=O,Gm=G. The code for the ant fourth from the right would be: H=G,T=-,Gl=G,Gr=Y,D=-,Gm=-.*

The largest colony used in this project had 95 workers which each needed a unique colour code. Before beginning the marking procedure a template of colour codes was devised using seven colours, see table 2.1, on the three body parts with the gaster being either split into left and right or a dot in the centre on top of a main colour. This generates more than enough combinations, using permutations there are 210 combinations of 7 colours on just 3 locations alone that could be used, i.e if only one colour was used on the gaster. However, some of the colours are difficult to distinguish when used together, for example red next to orange, and sometimes the colours markings are lost during the experiment through grooming therefore codes that are robust to the loss of one part must be used.

The combination of individually marked workers and a nest with a transparent roof meant it was possible to observe and track all members of the colony while they were inside the nest during the experiment. This makes it feasible to build a complete network of interactions between individuals.

Colour	**Code**
Blue	B
Green	G
Orange	O
Pink	P
Red	R
White	W
Yellow	Y

Table 2.1: *Codes for the different colours used in marking the ants*

2.2 Treatments

Each colony underwent two treatments: a control treatment and a famine relief treatment. The treatments were carried out at the end of October 2006, i.e. a month after being collected. The control treatment is intended to represent everyday conditions inside the laboratory when food is abundant. This was followed directly by a famine period where food was removed for 48 hours. At the end of the 48 hours the famine relief treatment commenced with fresh food provided to represent the appearance of a new source of food after a period of food shortage.

Three days prior to the control treatment, the colonies were provided with water, honey solution and dead *Drosophila* flies. Thus on the day of the treatment the conditions represented a typical day (under laboratory conditions at least) for the colony with no effect of starvation. Flies are provided as a source of protein which is utilized mostly by the queen and larvae [60]. At the start of the control treatment the flies are removed and two droplets of fresh honey solution (one part honey, ten parts water) were placed in a planchet ≈ 1 cm from the nest entrance as seen in figure 2-2. Immediately after the control treatment, the honey solution was removed for 48 hours. The famine relief treatment commenced at the end of this 48 hour period when a new planchet containing two fresh droplets of honey solution was provided. In both treatments the honey solution was provided in a planchet in the same location outside the nest ≈ 1 cm and at 90° from the entrance.

Recording for both treatments began when the first foraging ant to drink from the honey solution re-entered the nest. The nests were filmed from above, using a Panasonic NV-MX500B digital video camera, for three hours for both treatments. This generated 32 1.5 hour tapes to analyse, i.e. two per colony per treatment,

however we will see later that only a subset of these were used. As this species has seasonal behaviour which changes with time, [10], both treatments for all eight colonies were filmed within one week, using two cameras in parallel as shown in figure 2-2, to minimise any effect of time.

While the nests were being filmed, observers used the audio channel, AC, to record the colour codes of individuals drinking at the honey solution outside the nest. The observer states the colour code of the ant and whether they were starting or finishing drinking, for example "Red head green gaster begins drinking".

2.3 Data collection

The data required for this project are primarily feeding data, i.e. which ant donates food to which other ant, and spatial data, i.e. where each ant is inside the nest as a function of time. The obvious route to obtaining these data from the videos in this experiment would be to use automated tracking software to track each individual. Several options were explored including: software from Noldus called "Etho-vision Colour Pro" (as used in [38]); code written by Guy Blanchard for his PhD thesis [113]; ImageJ; and more recently ctrax (see [114]). Due to several reasons these automated tracking devices were not suitable. The main problem appears to be the number and density of individuals used in this project and that individuals often overlap one another inside the nest for substantial periods of time making individuals and their predicted paths difficult to distinguish, see figure 2-4.

It was decided that the tracking would be done manually using AntTracker a bespoke piece of software written by Andy Lulham [115]. This software plays back videos and allows the user to follow individuals with the cursor in real time, recording the position of the cursor on the screen. Inbuilt functions allow the user to record times and locations of behaviours. The drawback of using this software was that it could not playback at faster speeds. I overcame this issue by using VLC media player in conjunction with AntTracker as VLC allows the user to play back the video at a variety of slower and faster speeds.

Due to the large amount of time it takes to manually track an entire colony, data has so far been collected from four out of the eight available colonies for both

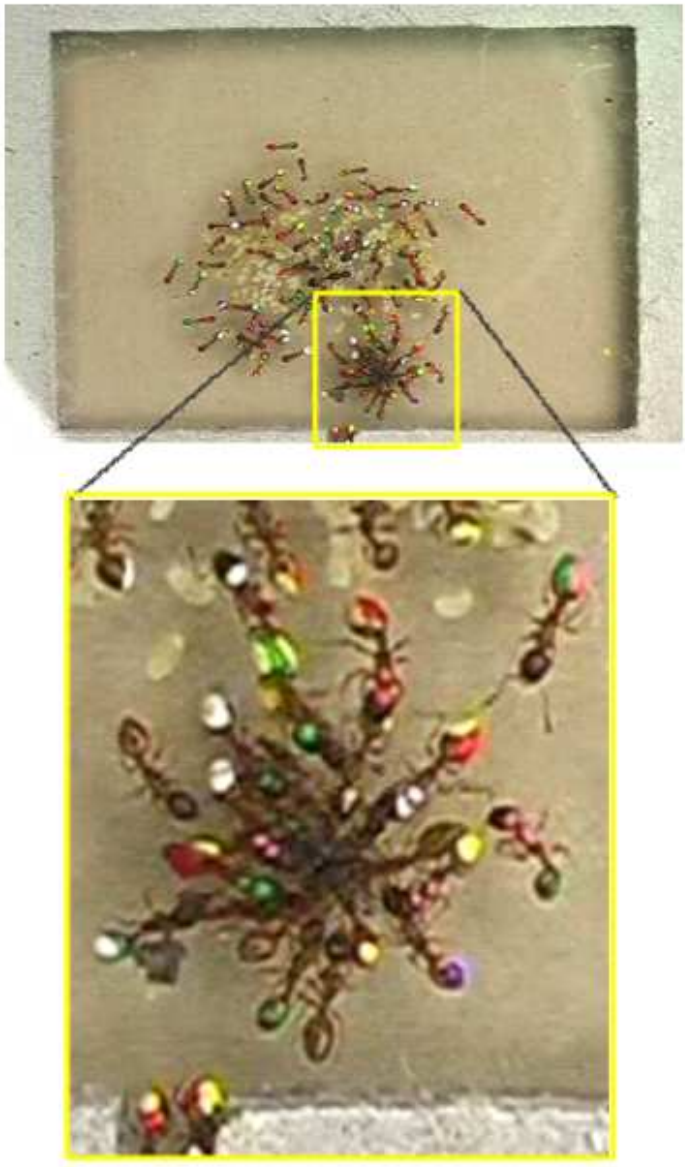

Figure 2-4: *An example of a situation where an automated tracking software is likely to fail in identifying individual ants. The inset shows an aggregation of ants around a returning forager. The workers crowd round the forager and climb over one another making it difficult to determine the outline of each individual.*

treatments; these were colonies III (number of adults in each colony including queen, N_C=42), IV (N_C=95), V (N_C=77) and VIII (N_C=49). These colonies were selected at random from the eight available. Data on the space use, feeding behaviour, brood items and drinking at the honey solution were collected for the first thirty minutes of each treatment in these four colonies. The decision to use only the first thirty minutes was made after observing the activity inside the nests during the famine relief treatment. During the first 15 minutes the activity levels in all four colonies appear high compared to that under the control condition. Then during the following 15 minutes the activity starts to decrease and return to that seen under control. Early analysis of the data, for example see figure 4-8, shows that thirty minutes was an appropriate length of time to analyse. Given the time it takes to manually track the ants analysing the videos for longer did not seem a valuable investment.

Statistical analysis was carried out in SPSS 14.0 and purpose built programs written in Fortran. Network analysis was carried out using UCINET version 6 [116].

2.3.1 Spatial data

As explained in Chapter 1, *Temnothorax albipennis* colonies have a strong spatial structure inside the nest which could potentially influence the transmission of food. To investigate whether this is true, information about the spatial structure, i.e. the space use of individual ants, is required. To determine each individual's space use I tracked every ant for the entire 30 minutes, using her unique colour code to identify her. I used AntTracker to record the x and y coordinates inside the nest cavity of the midpoint of her head every 60 seconds starting at $t = 0$ seconds, i.e. the start of the video. These data will be referred to as 'Spatial Point Samples', SPS. When an ant was outside the nest at the time of a SPS I recorded a location of $x = 100$ and $y = 100$ representing an arbitrary position outside the nest cavity. In AntTracker the frame is split into a 100×100 grid, so the x and y coordinates recorded are initially on a scale of 0 to 100 and are later converted into mm. PowerDVD v.4 and VLC media player were used to playback the videos at speeds faster than real time to reduce the time taken to follow an individual between recording the locations as this capability was not available in AntTracker at the time of the project. In total this process generated 31 data points per individual per treatment.

Figure 2-5 gives an example of the spatial data collected for one ant. It shows the amount of time spent inside and outside the nest in a.), where the worker was inside the nest at the spatial point samples in b.) and finally the shortest paths the worker took between these point samples in c.). These are minimum distances because they are straight lines between two consecutive spatial point samples, in reality the worker may have taken a longer route in the minute between two points. Therefore in Chapters 3 and 5 when I investigate speeds of workers they will be minimum speeds.

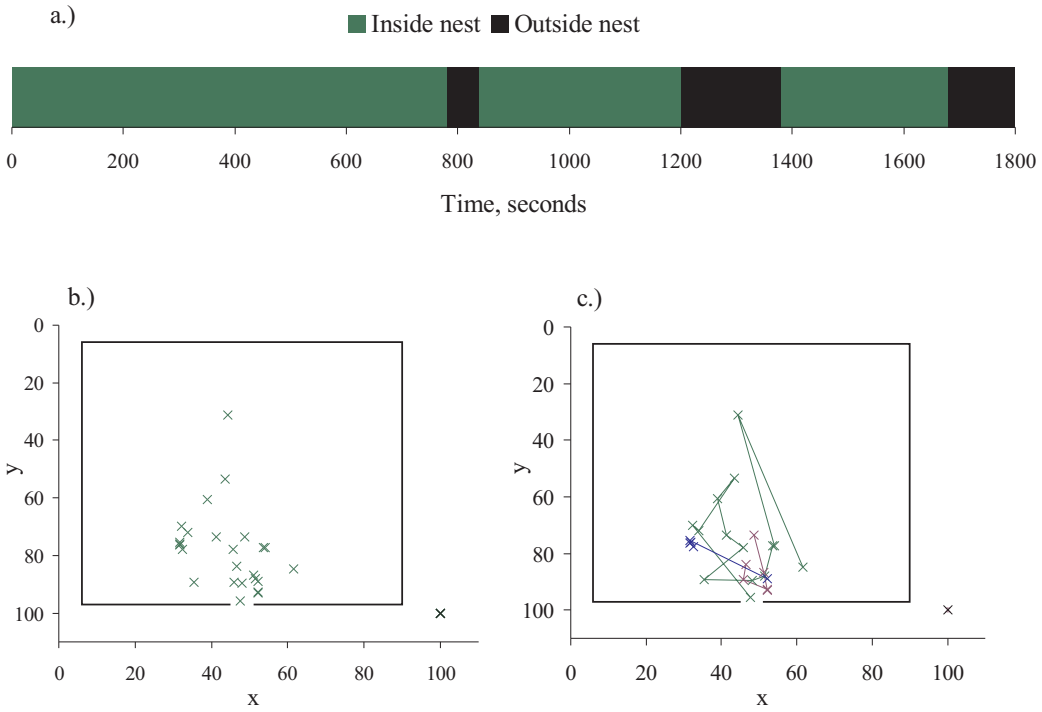

Figure 2-5: *An example of the spatial data collected for one ant. a.) A timeline representing when the ant was inside and outside the nest during the 30 minutes of a treatment. b.) The locations inside the nest that the ant was recorded at each minute over 30 minutes, i.e. the 31 SPS, the black outline represents the nest wall, points at (100,100) represent when the individual was outside the nest. c.) Locations as in b.) but joined with straight lines to show consecutive positions for bouts inside the nest, each colour represents a separate trip inside the nest.*

In order to aid the collection of the trophallaxis data and the spatial point samples during the famine relief treatment I also recorded the time and identity of the ant every time an individual entered the nest. This was only done for the famine relief treatment as activity was much higher and ants often entered the nest upside down obscuring their colour code.

Colony	**III**	**IV**	**V**	**VIII**
Number of ID labels	59	117	90	60
Dead ants	5	0	0	2
Ants never seen + unmarked ants which double up	12	10 + 8	6	4 + 2
Ants which enter and exit upside down in one treatment so not be identified	0	4	7	4
Total number in colony, N_c	42	95	77	48
Number tracked in both FR and CC	42	81	75	44
Number tracked in FR	42	86	75	47
Number tracked in CC	42	89	76	45
Number not tracked out of total	0	14	2	4

Table 2.2: *Number of ants tracked in each colony. FR = Famine Relief treatment, CC = Control treatment.*

Table 2.2 gives the number of ants in each colony and the number tracked. In some cases an individual was impossible to track during a treatment. This may be because the individual entered and then exited the nest and was upside-down the entire time so the colour code could not be obtained. The number of ID labels in table 2.2 is higher than the number of workers because an ID was assigned to each ant in the two treatments independently and was then matched up after tracking. This meant that if two ants were similar and could not be matched up, for example if there were two unmarked ants in a colony, extra ID labels were created.

2.3.2 Trophallaxis data

To obtain a complete trophallaxis data set each ant was followed for the 30 minutes whilst recording the start and end times for any trophallaxis events she was involved in. Start and end times were recorded at the resolution of one second, therefore unless otherwise stated one 'time-step' will mean one second. It is important to note here that this process was 'ant-driven' as opposed to 'event-driven', i.e. for each feeding event the donor and recipient were each detected independently while tracking separate ants as opposed to watching the video and looking for trophallaxis events. Using this 'ant-driven' method introduces a double-check as both a recipient and a donor for a feeding event have to be identified (i.e. cannot have a recipient without a donor) and thus improves reliability further. In section 2.4 I detail how I used an event driven process to verify the

data collected in this stage. The data collected in this ant-driven process will be referred to as the 'Initial Trophallaxis Data', ITD.

The presence of a trophallaxis event was determined by behavioural observations. Unlike allogrooming, where the ant performing the grooming may be oriented toward any part of the recipient's body, in a trophallactic exchange the donor is head to head with the recipient. The recipient might tap the donor on the body beforehand to solicit a donation and during trophallaxis there is a lot of antennal contact between the donor and the recipient (Ch.7 in [9]). When a trophallaxis event was detected, the role of the ant being tracked was recorded and could be either 'donor' or 'recipient'. The direction of food flow was determined by mandible position, the donor is recognisable by her characteristic open mandibles [79], and body posture of the workers involved [117]. This enables the correct assignment of donor or recipient role to the individuals participating in the feeding event.

In this project only the transmission of food inside the nest was of importance, so only the trophallaxis events occurring inside the nest were recorded. The nest cavity is all that can be seen on the video, as shown in figure 2-1, however occasionally an ant outside the nest will walk over the top of the nest and very rarely two ants will perform trophallaxis outside on top of the nest in view of the camera. This shows that whilst very rare, trophallaxis does happen outside the nest. These rare interactions will not be considered in this project for several reasons. Firstly from a practical point, the camera does not film the entire arena outside the nest so there may be some external trophallaxis events which are not possible to record. Secondly both ants involved in a trophallaxis event outside the nest are so called 'external ants' (ants which leave the nest) and therefore in this situation are likely to be foragers. The primary purpose of the trophallactic exchange between these two individuals is likely to be to pass on information to the recipient as opposed to the food itself. It is likely that the recipient will subsequently locate the honey solution herself and then bring food back to the nest. However even if the exchange was purely to transmit food both are external ants therefore if the recipient returns to the nest and donates, the food source would still be considered external as if she had drank from the honey solution directly.

Once all the members in a colony were tracked from one tape, the initial trophallaxis data for all individuals were amalgamated and separated into distinct trophallaxis events, i.e. recipients with their corresponding donor. This was achieved

using an algorithm written in Fortran to order the data by start time and then location. Once sorted the data were then grouped into distinct trophallaxis events based on proximity in time and location and correct combination of donor and recipient. Figure 2-6 shows an example of the output from the algorithm. For each trophallaxis event there is one donor and at least one recipient. There may be several recipients, as in event 2 in figure 2-6, where ant 14 was the donor and ants 26, 16 and 23 were the recipients. At this stage, if a reception event was present in the data with no matching donor (or vice versa) the algorithm highlighted the event and I went back to the videos and identified the missing donor and added the donation event to the data. This sorted and grouped version of the data is the 'Initial Trophallaxis Data', ITD, that is checked during the verification process outlined in section 2.4.

Ant ID	x	y	Role	Start frame	End frame	Event ID
9	54	80	2	975	1004	1
43	54	80	1	975	1004	1
14	48	86	2	1143	1153	2
26	49	86	1	1145	1183	2
16	48	85	1	1155	1170	2
23	48	84	1	1175	1184	2
37	56	92	2	852	869	3
11	55	92	1	852	868	3

Figure 2-6: *An example of the initial trophallaxis data, ITD, after it has been sorted by start frame and x and y coordinates to separate into trophallaxis events. 'Ant ID' denotes the identity of the ant, discernible from the unique colour codes. 'Role' is 1 if the ant is a recipient in that particular event and 2 if a donor. 'Event ID' is the ID given to each feeding event which must consist of one donor and at least one recipient.*

2.3.3 Brood data

The brood pile in *T.albipennis* can be considered as the biological centre of the colony [95]. As described in Chapter 1 the spatial structure of the workers is based on the brood pile. It is therefore useful to have information about the brood piles

of the colonies involved. The coordinates of every brood item inside each nest at the beginning and end of both treatments was recorded using AntTracker. Figures 2-7 and 2-8 show the positions of the brood items inside the nests of each of the colonies at the start and end of control and famine relief treatments respectively. The distribution of the brood items within the nests does not change greatly between the start and end of a treatment and also between treatments. From here on the brood pile is assumed to be static and any analysis which involves data regarding the brood pile utilizes the set of points taken at the start of the famine relief treatment.

2.3.4 Drinking data

From the spatial data collected and simply from observations during the experiment we know that several ants in each colony go outside the nest. To find out which of these ants are actually acting as foragers information about which individuals drink at the honey solution is required. The durations of these drinking events are used later in Chapter 6 to calculate the amount of honey solution transferred in a trophallaxis event.

Information was extracted from the audio channel, AC, to identify which ants were drinking at the honey solution outside the nest, when and for how long. The drinking times were obtained by listening to the AC in conjunction with watching the video to confirm the ID of the ant when it re-entered the nest. For each time an ant drank at the honey solution the ID, start time, end time and subsequently the duration of the drinking bout were recorded. When several ants are drinking simultaneously and starting and finishing in quick succession of one another it is quite difficult to keep track of every ant. Therefore the data-set collected from the AC for drinking times is not exhaustive as occasionally the start or end time for a drinking event has not been recorded. Table 2.3 shows the number of drinking events recorded on the AC and the number which had a start or end time missing. The number of ants that went outside the nest, external ants, in each treatment is a lot higher than the number of ants recorded drinking and the number of trips outside the nest they made was higher than the number of drinking events recorded. This suggests that more drinking events occurred which were not recorded on the AC. In Chapter 6 I will use the spatial information and the net food calculated to deduce which external ants, in addition to those

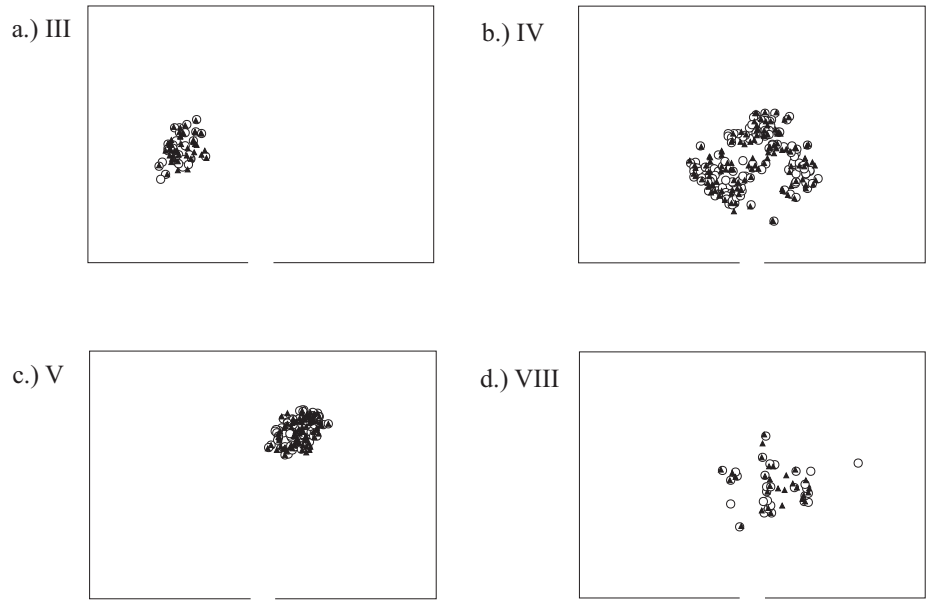

Figure 2-7: *Brood items at the start ∘ and end ▲ of the control treatment. The black rectangle represents the outline of the nest cavity, the gap indicates the entrance. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

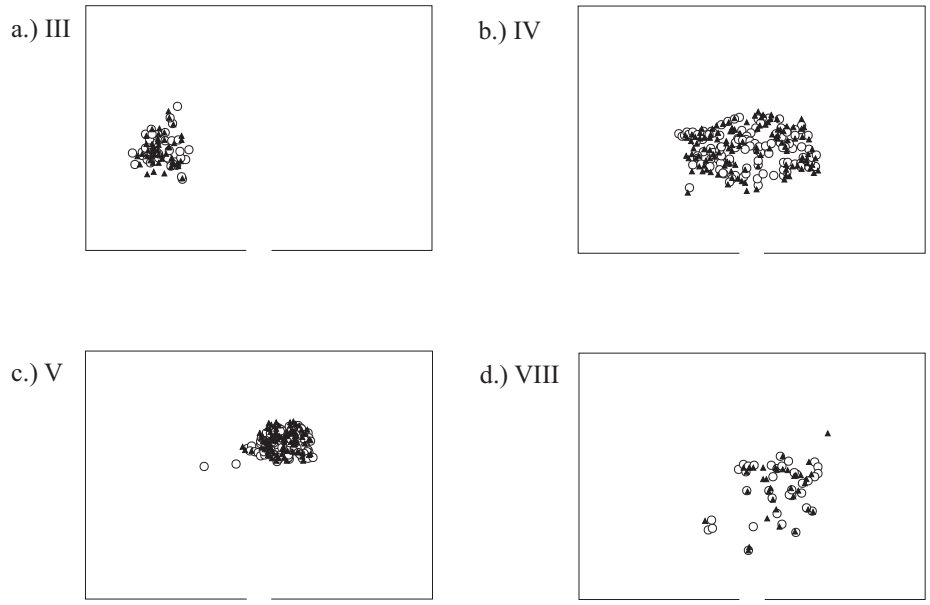

Figure 2-8: *Brood items at the start ∘ and end ▲ of the famine relief treatment. The black rectangle represents the outline of the nest cavity, the gap indicates the entrance. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

confirmed from the AC, were likely to have drank at the honey solution and acted as 'sources' providing food for the colony. For future experiments it may also be useful to film the ants drinking at the honey solution so a more complete data set can be obtained.

Treatment	**Colony**	**Drinking events**	**IDE**	**Drinking ants**	**External ants**	**Trips**
Control	III	7	0	5	17	39
	IV	6	3	6	40	85
	V	7	0	6	40	67
	VIII	4	2	3	25	60
Famine relief	III	21	6	7	13	38
	IV	26	9	7	25	61
	V	27	21	12	35	110
	VIII	45	5	8	13	39

Table 2.3: *Number of ants that drank from the honey solution in each colony. 'Drinking events' is the number of drinking events recorded on the AC. 'IDE' - Incomplete Drinking Events is the number of drinking events recorded that are missing either a start or end time. 'Drinking ants' is the number of ants recorded on the AC drinking at the honey solution. 'External ants' is the number of ants that ever went outside the nest in that treatment. 'Trips' is the number of separate trips outside the nest made by external ants.*

2.4 Data verification

During the early analysis of the initial trophallaxis data, ITD, it became apparent that a number of feeding events were either missing or had the incorrect ant recorded as the donor or recipient. Identifying and tracking every individual by hand is obviously a rather arduous task due to the reasons outlined in section 2.3. The first round of tracking to collect the ITD took 32 days with 262 hours tracked from the videos and was carried out by the two masters students, Benjamin Wulf and Thomas Klimek. Given the difficulty of the manual tracking the occasional mis-identification is inevitable.

However, as the network being analysed from this data represents an actual instance of a transmission event, as opposed to an average of social contacts, it is vital that the data set is as accurate as possible.

The errors in the ITD can be grouped into different types as defined in table 2.4. I designed a process to identify and semi-automate the correction of these

errors. I only carried out this verification process for the famine relief treatment and not the control because, due to the lower levels of activity during the control treatment, ants were easier to follow and trophallaxis events less likely to be missed or the incorrect ant recorded as a participant. For example, there were in total 110 feeding events recorded during the control treatment in comparison to 652 initially for the famine relief treatment, see table 2.5. In addition, the ants typically moved more slowly during the control treatment, see figure 3-6, and most feeding events were pairwise (one donor and one recipient) as opposed to multiple recipients feeding from one donor, see figure 3-4 in Chapter 3. The stages of this verification process are outlined below:

Acronym	**Name**	**Description**
ME	Missing Event	The feeding event is missing from the data entirely
RM	Recipient Missing	A feeding event with at least one recipient is present in the data but an additional recipient is missing (a missing donor will have been detected in the sorting stage, see section 2.3.2, likewise a missing recipient would have been detected if no other recipients were involved in the feeding event).
IAD	Incorrectly Assigned Donor	The feeding event is present in the data, however the donor recorded in the event is incorrect.
IAR	Incorrectly Assigned Recipient	The feeding event is present in the data, however one of the recipients in the event is incorrect.

Table 2.4: *Error types*

1. Minute samples of 'Apparent Trophallaxis Events':

To search for ME and RM errors, in AntTracker I recorded the location and number of ants involved in Apparent Trophallaxis Events, ATE, visible in the frame at 60 second intervals, for example see figure 2-9 a.) which shows the photo frame of the 13th minute of colony III under famine relief. There are three trophallaxis events visible circled in green.

2. Compare Apparent Trophallaxis Events, ATE, with Initial Trophallaxis Data, ITD:

To detect ME and RM errors I wrote an algorithm which searched the ITD to check that it contained each of the events I identified in the previous stage, the ATEs. Any ATEs highlighted as not present in the ITD were investigated on the videos and if deemed to be a true ME error were added to the trophallaxis data once the participating ants were identified, see figure 2-9 b.). Similarly for RM errors, if an ATE matched an event in the ITD but more recipients were recorded in the ATE than in the original event, the extra recipients were investigated and added to the trophallaxis data if necessary.

3. Identifying IAD and IAR errors:

To detect IAD and IAR errors I wrote an algorithm which, for each ant, searched the spatial point sample data, SPS, to confirm that it corresponded with the locations of the trophallaxis events that ant was recorded as involved in in the ITD. If the information from the spatial data did not correspond with the initial trophallaxis data a mis-identificantion error (IAD or IAR) had been detected.

Any of the trophallaxis events can be classified as one of two categories; those where the duration of the trophallaxis event crosses a spatial point sample and those which don't, for example see figure 2-10. Events which cross a SPS will be referred to as M1 events and those which do not cross a SPS will be referred to as M2 events. For example, as in figure 2-10, a trophallaxis event which started at $t = 30$ seconds and ended at $t = 95$ seconds crosses the point sample taken at $t = 60$ seconds and is therefore an M1 event. A trophallaxis event that started at $t = 143$ seconds and ended at $t = 172$ seconds does not cross the sample points $t = 120$ seconds or $t = 180$ seconds so is an M2 event.

M1 events were flagged if the location of the trophallaxis event that an ant was recorded as participating in was more than 4mm, i.e. two bodylengths, away from her location at the time of the spatial point sample that the event crossed. 4mm was chosen as large enough to be a definite error but small enough to not to falsely highlight cases where the correct ant was recorded but with small errors in the recorded locations.

M2 events were flagged by looking at the distance between the closest spatial point sample to either the start or the end time of the trophallaxis event. For example the location recorded for the M2 event in figure 2-10 would be compared to the locations for the donor and recipient in the event using their SPS at $t = 120$ seconds and $t = 180$ seconds.

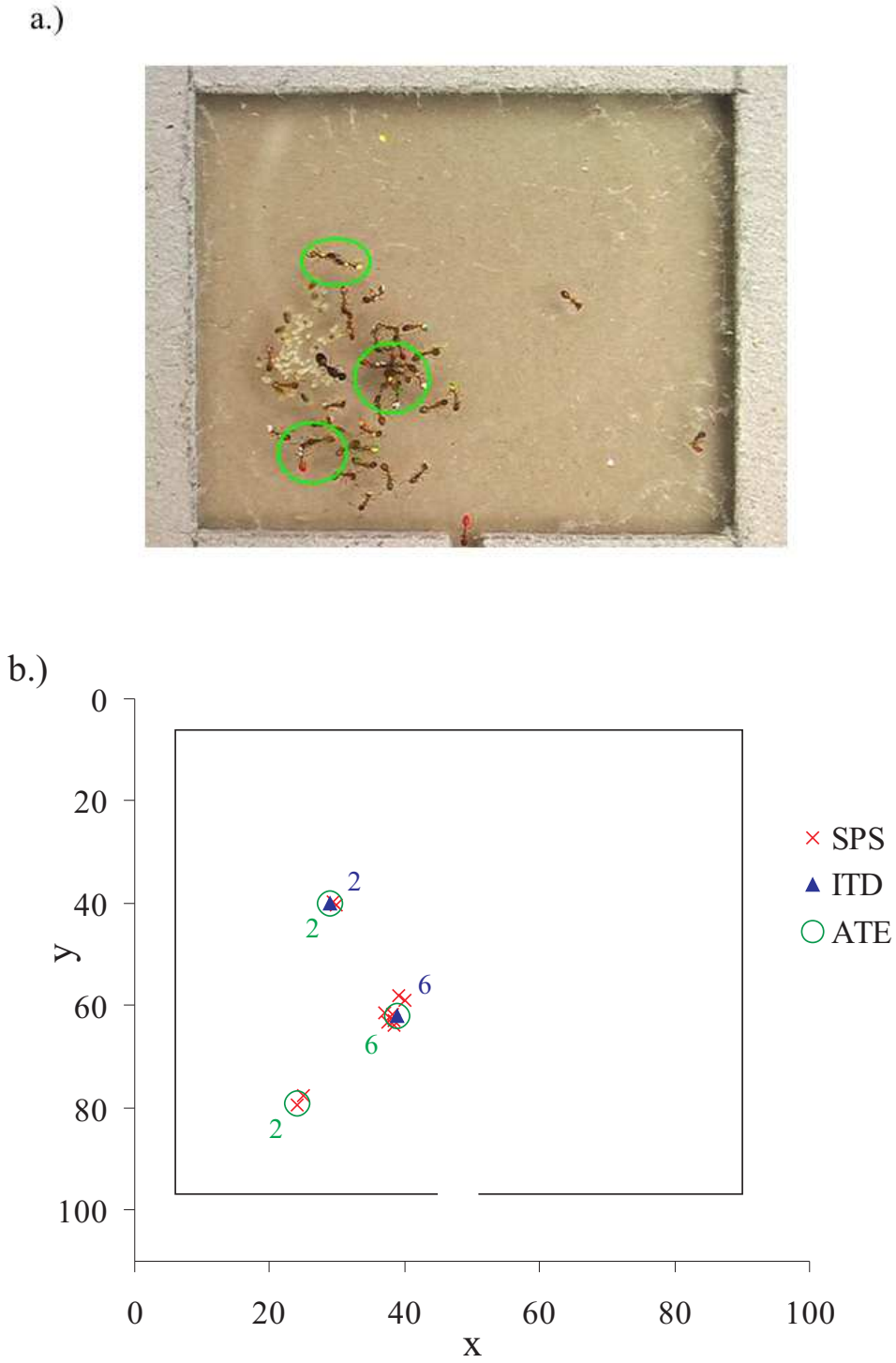

Figure 2-9: *An example of how Missing Event, ME, and Recipient Missing, RM, errors are detected and resolved. a.) The photo frame of the 13th minute sample of colony III under famine relief, three Apparent Trophallaxis Events, ATE, are circled in green. b.) Comparing the ATE data with the initial trophallaxis data, ITD, and spatial point samples, SPS, this highlights that the ATE in the lower left has not been recorded in the ITD so will be added. Numbers in green represent the number of ants participating in the trophallaxis event for the ATE, while blue numbers represent the number of ants in the event recorded in the ITD.*

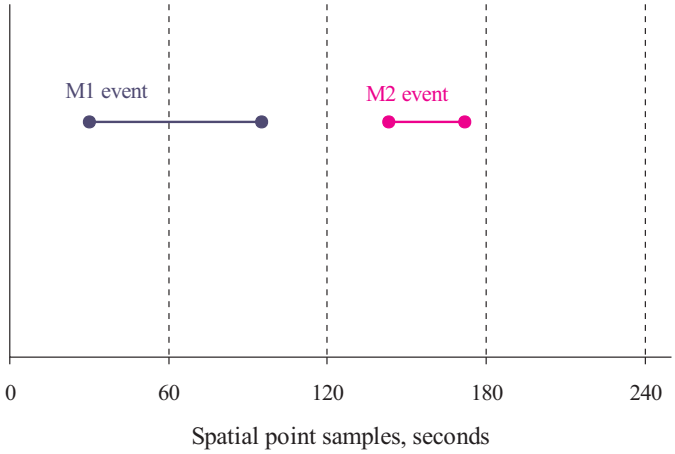

Figure 2-10: *An example of M1 and M2 trophallaxis events. The M1 event crosses the SPS for* $t = 60$ *whereas the M2 event crosses neither the SPS at* $t = 120$ *or* $t = 180$.

If the distance between the trophallaxis event and either of the SPS was greater than 4mm or both SPS were not within 10mm of the trophallaxis event, or if the distance between the SPS and the trophallaxis event was greater than the calculated distance the ant could have moved based on an minimum average speed calculated for each ant, then the M2 event was flagged. This minimum average speed was based on straight lines between SPS as shown in figure 2-5 c.).

This procedure flagged a lot more M2 events than M1, however often many of these were falsely flagged, i.e. an event was flagged when actually the correct ant was recorded. These were quickly resolved by re-examining the videos and confirming the ID of the ants involved in the event. For both M1 and M2 flagged events ants that were within 5mm of the location of the trophallaxis event and not already recorded as participants of the event were highlighted as possibilities for the correct participant.

4. Checking flagged M1 and M2 events:

The next step was to work through the list of flagged M1 and M2 events with the corresponding list of possible correct ants and look on the videos to confirm the actual ant participating in the events and update any necessary changes in the trophallaxis data.

5. Confirming "Ambiguous" events in the original data:

In each colony there were a small number of events from the first round of tracking where it was not easy to identify which ant was the donor. These events were labeled as "Ambiguous" in the original data and no role was assigned to the participants they were therefore not included in the ITD. I referred back to the videos for these events and made attempts at assigning the correct roles. As the roles were not immediately discernible it is possible that my attempts were not always correct.

Table 2.5 shows the number of events in the ITD and the number of events added through this verification procedure. In one colony the number of events nearly doubles showing that investing the time in verifying the data was worthwhile.

Of course there are still gaps in this process where events may have been missed entirely, for example, an event that was missed in the first round and the duration of which did not cross one of the 60 second samples would not be detected (i.e. an ME M2 event). However, I feel that such missed events are rare given that the videos have now been searched at least twice, firstly using the initial 'ant driven' procedure and then using the 'event driven' procedure outlined in this section. Therefore the cost of designing and carrying out a process to detect and correct these rare events would be large in comparison to the benefits gained.

Colony	**III**	**IV**	**V**	**VIII**	**Total**
Number of pairwise events in ITD	80	208	269	95	652
Number of flagged M1 events	7	18	25	12	62
Number of flagged M2 events	6	42	73	9	130
Number of Ambiguous events in original data	40	23	13	12	88
Number of events added from ATE and M1M2 processes	35	119	8	12	174
Final number of pairwise events	155	350	290	119	914

Table 2.5: *Events added to or corrected in the initial trophallaxis data for the famine relief treatment by the verification process*

2.5 Data structure

Along with the spatial data collected for each ant, shown in figure 2-5, the information gathered during the data collection process has been stored in an arrangement of time-lines representing the various aspects of the transmission process.

An example of the first three of these time-lines for two ants are shown in figures 2-11 and 2-12. Storing the information in this way facilitates the processing and analysis of what is otherwise a fairly complex data set. The data-sets arranged into time-lines are as follows:

Entrances : Using the entrance data collected in 2.3.1, for each ant a time-line of when they entered the nest can be constructed. This can be used to determine the bouts of each forager, i.e. trips to the honey solution, returning to the nest and donating and leaving the nest again.

Donations : Using the trophallaxis data a time-line of when each ant is donating food can be made. Entries are zero when the ant is not donating and 1 when donating.

Receptions : Using the trophallaxis data a time-line of when each ant is receiving food can be made. Entries are zero when the ant is not receiving and 1 when receiving.

Donors : When an ant is receiving food, they can only be receiving from one donor at a time. This timeline stores a zero when the individual is not receiving and stores the ID of the donor in each timestep the individual is receiving for.

These timelines are used in Chapter 6 to calculate the net food each individual received during both treatments.

The remainder of this thesis will explore resource distribution using various aspects of these time-lines and the spatial data. The following chapter presents my initial observations made while carrying out the tracking and early analysis. These observations highlighted that the distribution of resources in the colonies could be looked at from three overlapping perspectives: how the distribution progresses as a function of time; how it is arranged spatially inside the nest; and finally the amounts of food distributed inside the nest along pathways to individual workers. These three perspectives form the basis of the final three chapters.

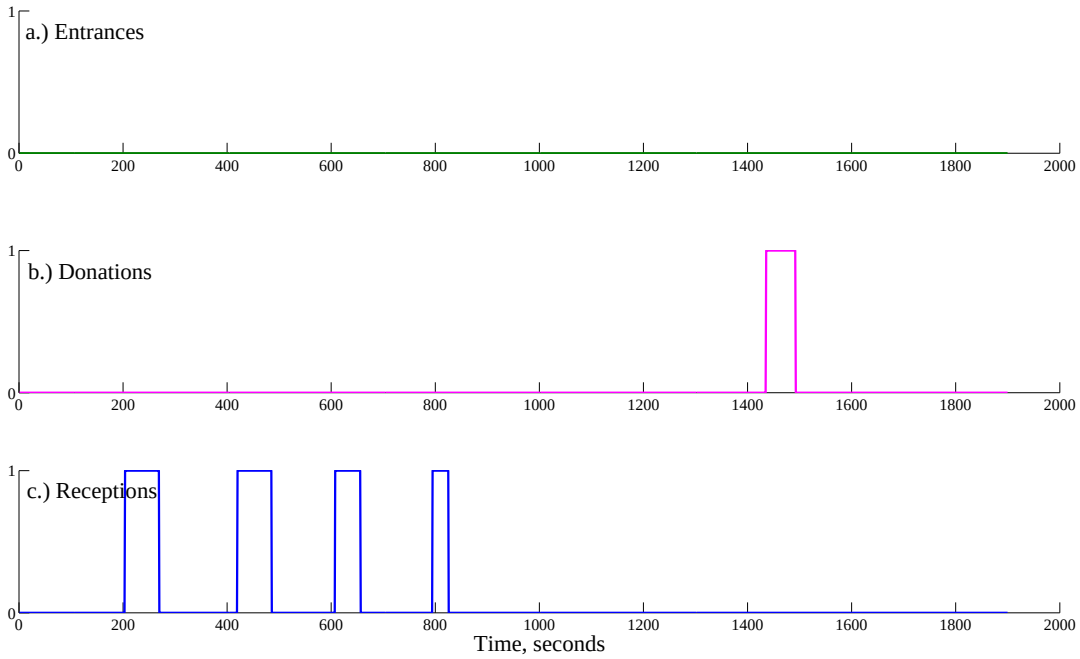

Figure 2-11: *An example of a set of timelines for an internal ant. This is ant 22 from colony VIII. a.) Entrances, b.) Donations, c.) Receptions.*

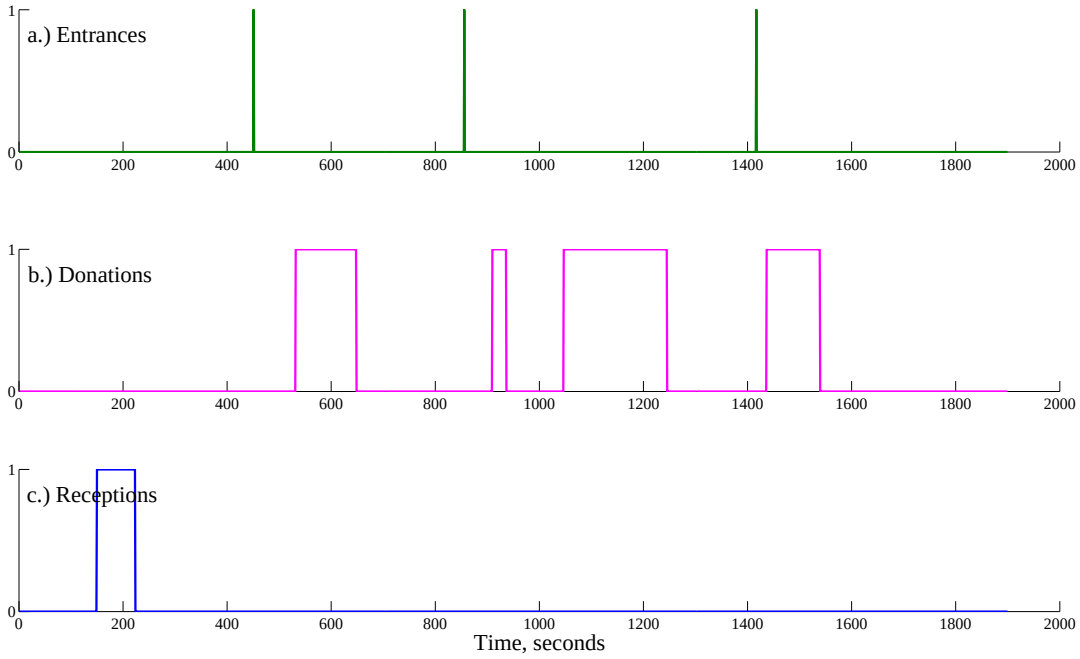

Figure 2-12: *An example of a set of timelines for an external ant. This is ant 6 from colony VIII. a.) Entrances, b.) Donations, c.) Receptions.*

Chapter 3

Gross responses to famine relief

The aim of this chapter is to outline the gross responses to famine relief seen in the four colonies as these guide the direction of the investigations presented in the following three chapters. Most of the results presented in this Chapter fall under the first two objectives set out in chapter 1: A - to compare rates of feeding between the two treatments and how faster feeding is achieved under famine relief; and B - to determine whether spatial structure is maintained during famine relief. These features include a comparison between treatments of feeding in groups, speed of internal workers and brood coverage. The remainder of the results in this chapter do not fall within the main objectives and generally relate to smaller observations or analysis which are relevant to this study including whether the queen receives food and the variation in colony demography.

3.1 Inter-Colony variation

It was evident from the start of the experiment that there is some variation in properties among the four colonies. Variation is present not only in the colony size, N_C, which ranges from 42 to 95 workers including the queen, but also in the layout, size and density of the brood pile. Figure 3-1 shows photographs of the nests of each colony from which the brood piles are visible. Figures 2-7 and 2-8 also demonstrate this graphically by showing the location of the brood items recorded at the start and end of both treatments. Colonies III and V have brood piles that are relatively small, homogeneous and approximately circular. Colony

V's brood pile is the most dense and furthest from the nest entrance. Colony IV has a very large brood pile, the edge of which is close to the nest entrance. The brood pile of colony VIII is relatively scattered in comparison to those of the other colonies. Table 3.1 shows the number of brood items and area covered by the brood in each colony. This area is calculated by forming a convex hull around the coordinates of the brood items, see Chapter 5 for more details on how convex hulls are calculated and used.

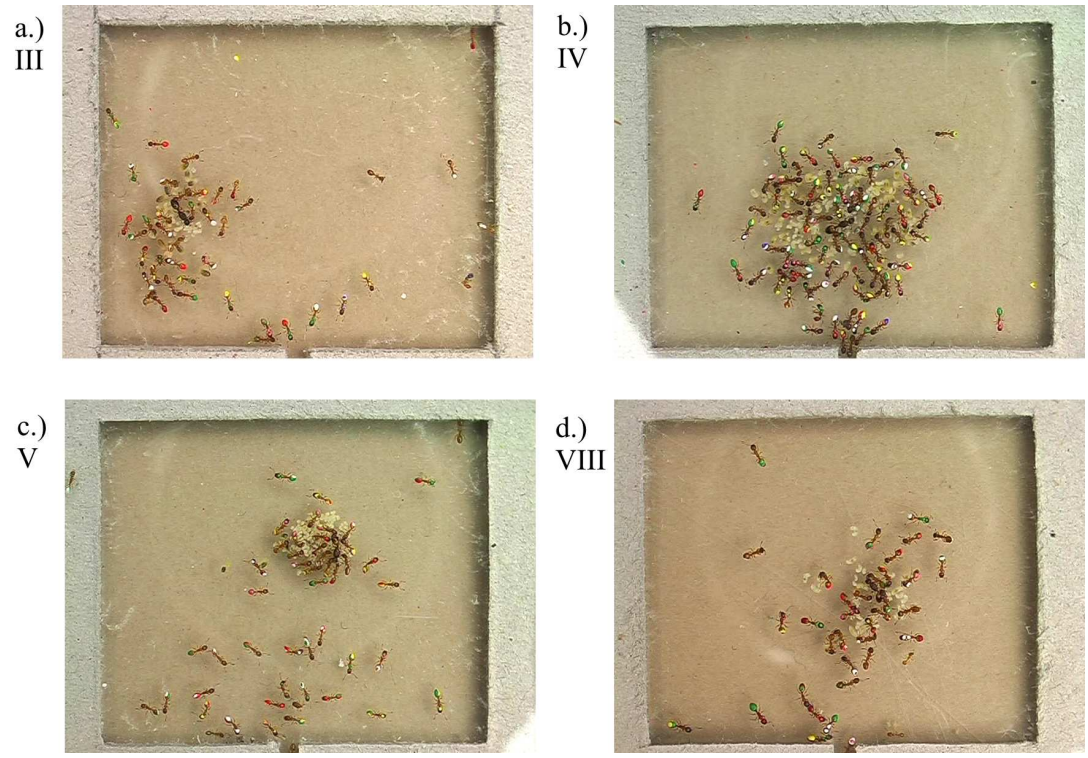

Figure 3-1: *Still-frames of each colony taken from the videos highlighting the variety in location, density, size and shape of brood piles. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

In the context of transmission of liquid food, which primarily involves adult workers and does not directly involve the brood items, the disparity of the brood piles may seem unimportant. However, the location and size of the brood pile may affect the distribution of food from two perspectives. Firstly from a geometric perspective: the area available for feeding to take place in may be affected, this is most prominent in colony IV where the edge of the brood reaches very close to the nest entrance. Meanwhile from a demographic perspective the number of brood items in relation to the number of workers would certainly affect the proportion of the colony that is involved in brood care as their primary task. It is also noticeable that there is a difference between colonies in the types of brood

items present; colonies III and IV both contain the smaller eggs and several larger darker larval stages, while V and VIII consist of mostly eggs and small larvae with a very small number of the larger larvae. At this magnification small larvae and eggs are indistinguishable. Figure 3-2 shows the differences in the ratios of brood items to workers and large larvae to workers between the four colonies. Colony V has the highest brood to worker ratio which means that each worker is potentially looking after a higher number of brood items than in the other three colonies. However, colony V has the lowest larvae to worker ratio which shows that most of colony V's brood pile consists of eggs or small larvae which require less care than the larger larvae, see for example "domains of care" in [96].

The differences in the numbers of workers in each colony are important as these could also affect how many workers are allocated to the various colony tasks. While the brood items are immobile and easy to collect it is possible that the reason the smaller colonies, III and VIII, have fewer workers is because some were missed during collection from the wild. However, the colonies used in this project were collected at the end of September; at this time of year the colonies are preparing for winter and most of the workers are inside the nest and therefore easy to capture [10]. This, in combination with the fact that the collectors waited for returning ants outside the nest after collecting the ants inside, makes it unlikely that a large number of workers were missed during collection. In addition, the colonies were left for a month in the laboratory to acclimatise, if a number of foragers were missed from the smaller colonies this period allows them to adjust task allocation among the remaining workers.

Given that there is a difference in colony size, the two larger colonies are close to twice the size of the two smaller colonies, how is this likely to affect the food distribution process? Perhaps there will be an economy of scale, whereby the larger colonies are more efficient at distributing the food to workers inside the nest. Such economies of scale are seen in other distribution processes in biology, e.g. see [118], and in eusocial insects, e.g. [119]. Alternatively each colony may adopt a different strategy that is most appropriate for their own colony demography.

3.2 Trophallaxis with multiple recipients

Typically the transmission of liquid food, trophallaxis, is depicted as a pairwise interaction between a donor and one recipient as seen in figure 3-3 a.), and also

Colony	**III**	**IV**	**V**	**VIII**
Number of workers + queen	42	95	77	48
Number of brood items (no. eggs and small larvae + no. large larvae)	43	116	115	36
Approx. number of large larvae	12	29	3	4
Distance from centre of brood to nest entrance, mm	17.19	13.89	20.60	14.86
Distance from edge of brood to nest entrance, mm	12.67	9.03	18.05	6.49
Area covered by brood pile, mm^2	35.8	111.3	33.7	102.5
Density of brood pile, brood items per mm^2	1.20	1.04	3.41	0.35

Table 3.1: *Brood properties of the four colonies. Density is calculated for the area within the convex hull formed around the brood items.*

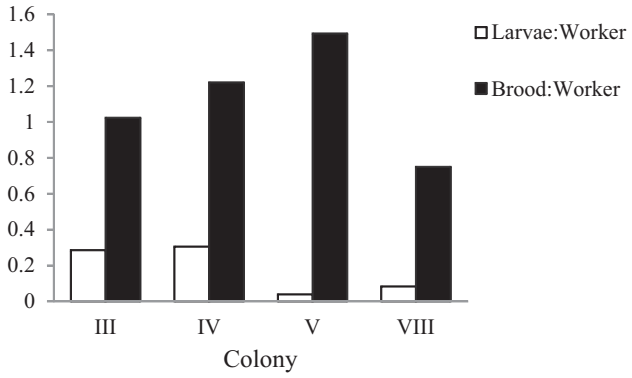

Figure 3-2: *Ratios of all brood items to workers, black bars, and large larvae to workers, white bars, in all four colonies.*

see p.258 in [9]. From watching the videos of this experiment it is apparent that under the control treatment most of the feeding is carried out in pairwise interactions as expected. However under the famine relief treatment, when the workers are starved and the need for food is more urgent, many of the feeding events occur with multiple recipients receiving from one donor simultaneously forming a 'rosette' of recipients, as seen in figure 3-3 b.). This arrangement has previously been observed in fire ants, *Solenopsis invicta*, where the maximum number of recipients feeding from a donor simultaneously rises from 3 to between 2 and 8 after a period of starvation [79].

Figure 3-3 shows photographs of these two feeding arrangements. It may seem difficult to discern from the second photo which individual is the donor so it is important to note that much more information about which ant is the donor and when each recipient is actually receiving can be gleaned from a video as opposed

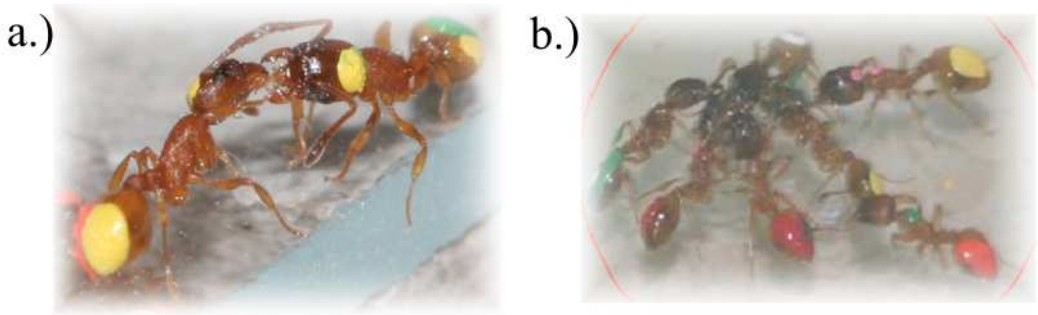

Figure 3-3: *a.) A pair-wise trophallaxis interaction. The ant on the right is donating liquid food to the ant on the left. In this example the donor is recognisable from her open mandibles and the body posture of the recipient, and b.) A rosette trophallaxis interaction. The ant at the top of the photo with a white marking on the gaster is the donor.*

to a photograph or still-frame. In addition to determining which individual is the donor it is also apparent that not all the ants in figure 3-3 b.) are receiving from the donor. For example the two ants furthest to the right in the photograph are not close enough to the donor to be receiving but are clearly approaching the rosette.

The proportion of receptions in rosettes is higher under the famine relief treatment compared with under control in all four colonies, see figure 3-4. In contrast, under the control treatment, in colonies IV, V and to some extent VIII, pairwise receptions appear to dominate, whereas in colony III they occur in roughly equal amount to simultaneous receptions in a rosette. A Chi-squared test was performed to determine whether these differences between the treatments are statistically significant, see table 3.2. An "expected" frequency of 50:50 is simply used to test whether statistically one type of feeding occurs more than the other and whether this changes between treatments. Under the control treatment the ratios of reception formations in colonies IV and V are significantly different from 50:50. Colonies III and VIII under control do not show a significant difference from a 50:50 ratio of pairwise and simultaneous receptions. Under the famine relief treatment the ratios of are significantly different from 50:50 in all four colonies and therefore confirm that significantly more of the receptions occur in rosettes than pairwise. The combination of the two chi-squared tests for each colony shows that the ratio of reception formations changes from either 50:50 or pairwise dominant under the control to rosette dominant under the famine relief treatment. Feeding in rosettes is a mechanism for distributing food to ants

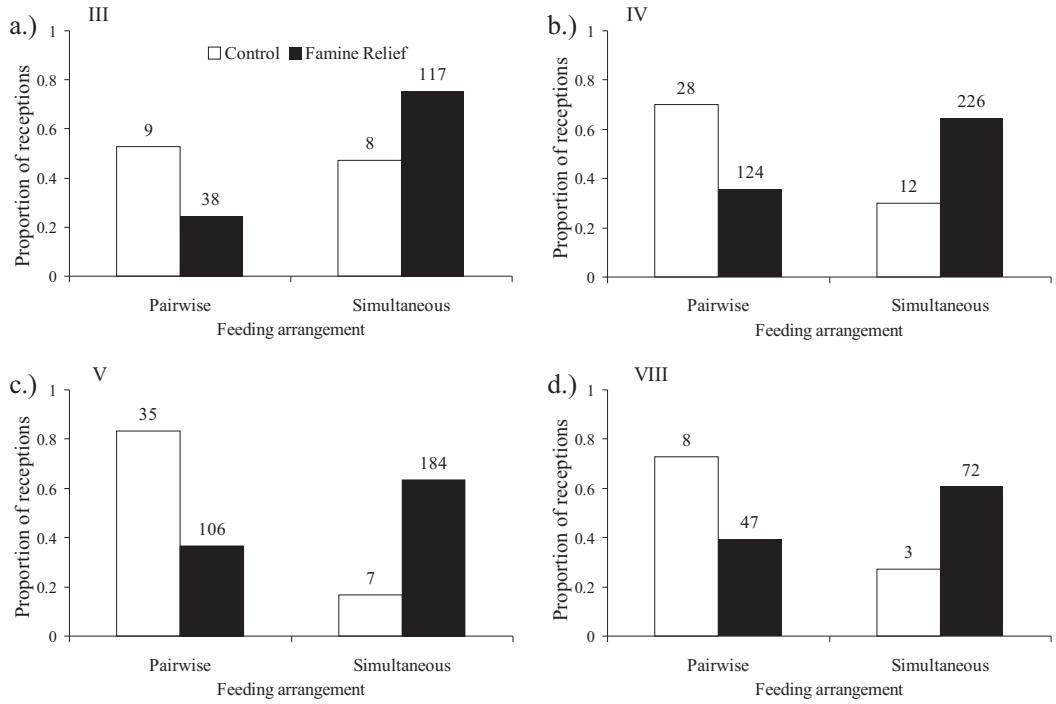

Figure 3-4: *Proportions of receptions in pairwise compared with simultaneous (in a rosette) trophallactic interactions. Numbers above bars give the actual number of receptions in each case. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

rapidly during famine relief compared to feeding one ant at a time. Figure 3-4 also shows the number of receptions for each case. This number is always much lower under the control compared with under the famine relief treatment because there are much fewer feeding events during the control which is also likely to cause the variation between the four colonies.

Colony	**III**	**IV**	**V**	**VIII**
Control	0.058	**6.400**	**18.667**	2.273
Famine Relief	**40.265**	**29.726**	**20.979**	**5.252**

Table 3.2: *Chi-squared values for number of pairwise and simultaneous receptions under control and famine relief treatments with an "expected" ratio of 50:50. Entries in bold indicate cases where the proportion is significantly different from 50:50, i.e. chi-squared values that exceed the critical value of 3.841 with 1 degree of freedom at the* $\alpha = 0.05$ *significance level.*

3.3 Frenzy behaviour under famine relief

On comparing the videos of the two treatments, consistently across all four colonies there appears to be a qualitative difference in the rate of movement and brood coverage between the control and the start of the famine relief treatment.

Under control, the colonies appear to be in a quiescent state for the majority of the time, with many of the workers completely stationary. This corresponds with previous findings which show that ants are inactive or resting for up to 70% of the time [24]. In contrast during the famine relief treatment the workers are sedate at the very start of the video but once the first foragers start entering the nest the activity levels soar. The workers abandon the brood and move rapidly towards the area between the brood pile and the nest entrance often flocking around an incoming forager in what appears to be a "feeding frenzy". Figure 3-5 shows a snapshot of this feeding frenzy in each of the four colonies under the famine relief treatment and highlights the difference between the typical arrangement of workers in figure 3-1. The area between the edge of the brood pile and the nest entrance is where many of the feeding rosettes form and from now on will be referred to as the 'arena' shown as white ellipses in figure 3-5 (but are defined with more detail in Chapter 5). These periods of heightened activity occur within the first ten minutes of the famine relief treatment in all four colonies and roughly last between 2 and 5 minutes. During the control treatment periods of heightened activity only occur much later (after at least 20 minutes if at all) and it does not appear as though any actual trophallaxis events are occurring during these periods. Given that the workers are not particularly hungry during the control treatment it could be that something other than an incoming laden forager is triggering such frenzies, for example perhaps the ant entering the nest has inadvertently picked up an unfamiliar odour from outside the nest. In contrast it is clear that under the famine relief treatment the increase in activity and abandonment of the brood pile during these periods is triggered by a forager returning to the nest to donate food.

Figure 3-6 illustrates the overall increase in activity under the famine relief treatment compared to under the control treatment by showing the increase in 'straight-line speeds', see section 2.3.1, of internal workers in all four colonies. The straight-line speed, SLS, is calculated for each ant by taking the sum of the distances, D_{ij}, between each pair of consecutive spatial point samples, SPS, inside the nest and dividing by the total number of SPS inside the nest, N_{in}, minus

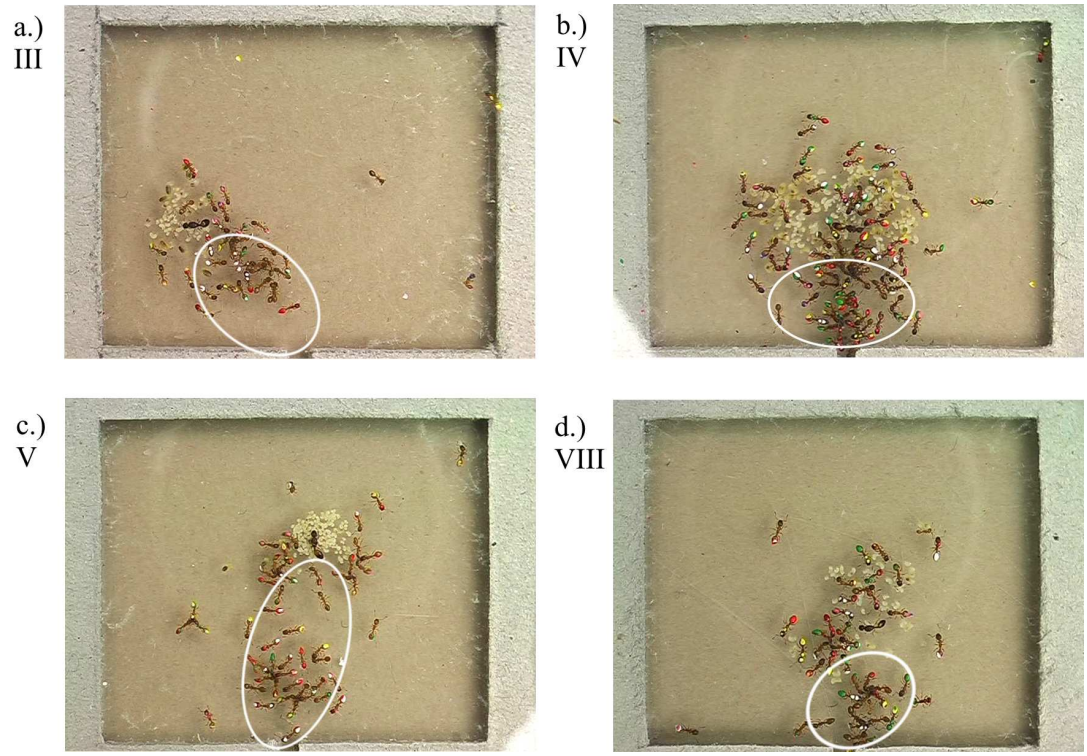

Figure 3-5: *Still-frames of each colony taken from the videos highlighting the feeding frenzies during the famine relief treatment. The white ellipse in each case roughly highlights the 'arena' between the nest entrance and the edge of the brood where a large amount of the feeding activity takes place. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

1 (i.e. 30 for internal ants) multiplied by 60 to get a speed in millimetres per second,

$$\mathrm{SLS} = \frac{\sum_{\substack{1<i<30 \\ 2<j<31}} D_{ij}}{(N_{in} - 1) \times 60}. \tag{3.1}$$

Internal workers for each treatment are those which did not leave the nest for the duration of that treatment. Workers that left the nest, external workers, are not included in this analysis as we have not recorded the exact times that they left the nest (and under the control treatment, the time they entered the nest). In addition, some external ants, particularly under the famine relief treatment, were only inside the nest to donate food during which time they were stationary. Colonies III, IV and VIII show similar speeds of internal workers under control and all show an increase under the famine relief treatment. Colony IV only shows a small increase under famine relief, but the movement of the workers in this colony may be restricted by the large brood pile. Colony V has a much

higher median speed under both treatments and little increase during the famine relief treatment. The large distance between the edge of the brood and the nest entrance in this colony may make it possible or even necessary for workers to move faster inside the nest. The biological importance of such frenzy behaviour with increased speeds of workers is likely to be that it increases the contact rate between individuals and therefore the efficiency of resource transfer to prevent starvation. The efficiency of receiving food (resource transfer) is addressed in Chapter 4.

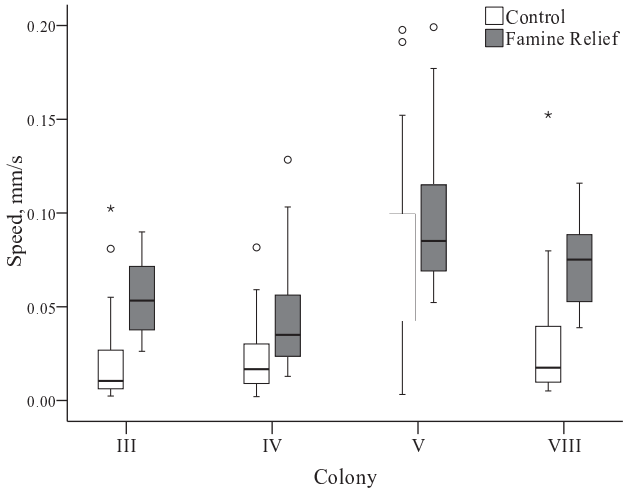

Figure 3-6: *'Straight-line speeds' of the internal workers in each colony under the famine relief treatment compared to under the control treatment.*

3.3.1 Brood coverage

While more quantitative analysis of the number or proportion of workers on the brood pile will come in Chapter 5, it is important to point out that during these feeding frenzies the brood pile can be left completely unattended by the workers and even, for a brief period, the queen. As an example, in figure 3-5 the brood piles of colonies III and V have virtually no workers on them compared to the level of coverage seen in figure 3-1. Figure 3-7 shows the proportion of tracked workers on the brood pile as a function of time for each colony. This was calculated using the convex hull formed around the brood items and summing how many of the workers' spatial point samples, see section 2.3.1, were inside the convex hull at each minute. It is clear from the figure that the brood is left unattended during the famine relief treatment between $t = 8$ and $t = 12$ minutes in colony III and

between $t = 5$ and $t = 9$ minutes in colony V. In colony IV the brood pile is never completely abandoned, however coverage is lower during the famine relief treatment than most of the control. The brood pile in this colony covers such a large area that it limits the area available for feeding between the pile and the nest entrance and probably forces several workers to remain on the brood at all times. In colony VIII the proportion of workers on the brood appears similar under the two treatments. As mentioned previously the brood pile in this colony is particularly scattered, this means that a convex hull drawn around the brood items is large and incorporates a lot of area that does not actually contain a brood item. This makes it difficult to discern from the spatial data alone which individuals are specifically concerned with brood care as several workers may be within the convex hull but not actually near a brood item. It appears that as the brood items are so scattered there is no clear abandonment of the brood as seen in the other colonies but there is a shift towards the nest entrance as illustrated in figure 3-5. This abandonment of the brood pile and crowding in the arena indicates that under the conditions imposed during the famine relief treatment adherence to spatial fidelity zones loosens. Figure 3-7 also shows that there are more ants outside the nest during the control treatment. This is an interesting result given that this species is expected to recruit a large number of nestmates to a food source particularly after a famine [111]. Why this occurs will be addressed further in Chapter 6 while individual space use is explored in Chapter 5.

3.4 The queen

Temnothorax albipennis is a monogynous species, with one queen to each colony. As the queen ensures the onward survival of the colony by producing eggs it is reasonable to ask whether she is involved in the process of transmission of food during famine relief. Perhaps special care is taken to feed her first, or on the contrary, perhaps she is fed much later so that there is time for the workers to test the quality and safety of the food. In two of the colonies under the famine relief treatment the queen does not get fed at all in the first 30 minutes and in the other two colonies the queen is fed once by an internal ant (IV, $t = 382$ to $t = 389$, seconds) and three times by three different foragers (V, $t = 657$ to $t = 789$; $t = 856$ to $t = 886$; and $t = 935$ to $t = 950$ seconds). None of the four queens receives food during the first 30 minutes of the control treatment. In this experiment, the aim was to examine the transmission of the liquid food that

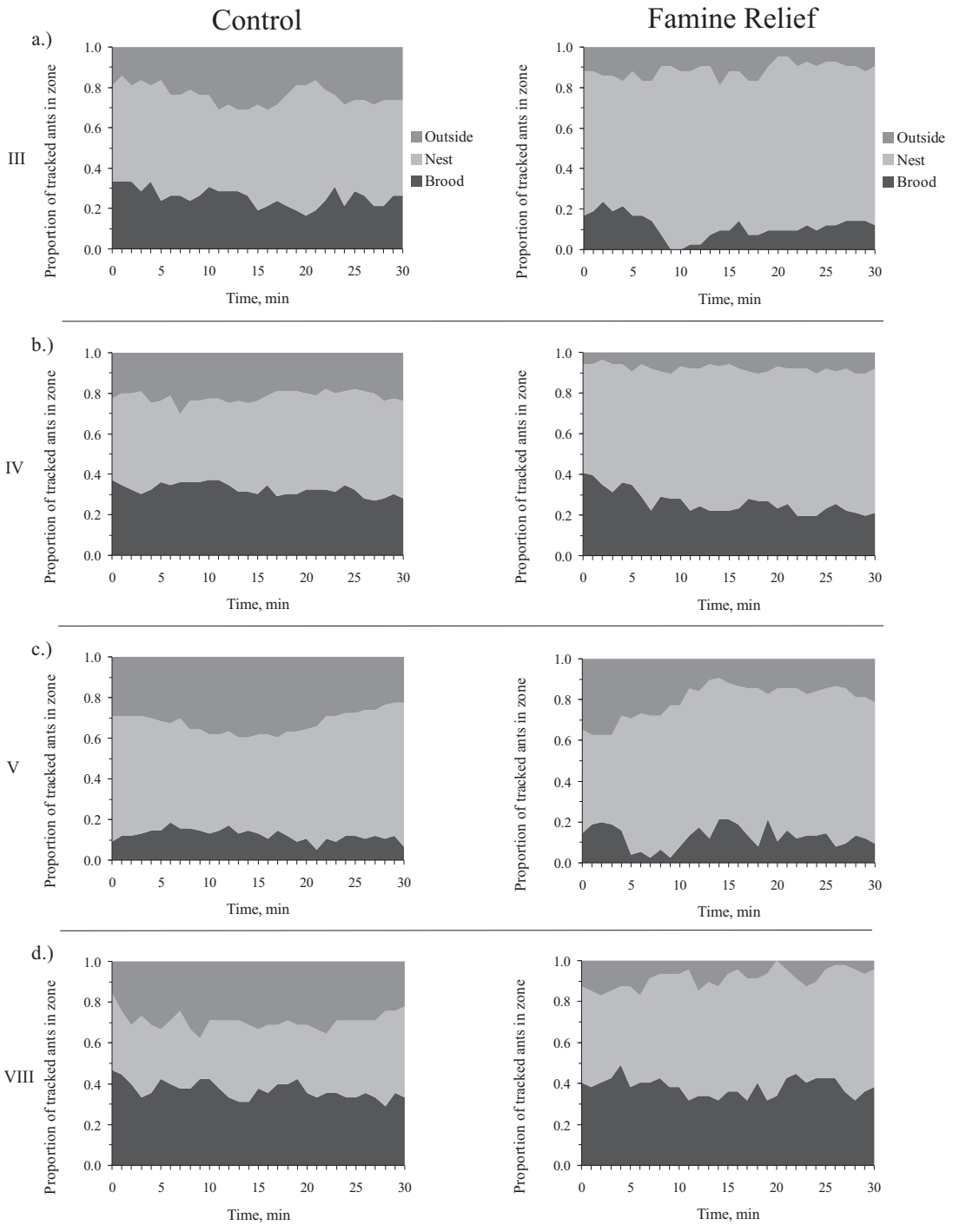

Figure 3-7: *Proportion of tracked ants on the brood, in the remainder of the nest and outside the nest as a function of time. Control treatment is shown in the left column, famine relief treatment is shown in the right column. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

comprised of a honey solution providing carbohydrates to the colony. The main nutritional requirement for the queen is for protein in order for her to produce new eggs [60]. Therefore, as the queen has little need for carbohydrates it makes sense that she does not play a big role during the transmission of the liquid food used in this experiment.

3.5 Summary

This chapter has outlined initial observations from watching the videos and early data analysis carried out. These observations guide the direction of the work presented in Chapters 4, 5, and 6. We will bear in mind throughout these chapters that the four colonies used exhibit variation in their demographic and geometric features and that these differences may influence the food distribution process. While consistency among replicates is generally desirable in any experiment, in this case differences between the colonies and their behaviours should not be considered as a negative outcome. In this chapter I have highlighted several differences between the colonies, including number of workers, size, shape, location and density of brood pile and coverage of the brood. They highlight that each colony may be in a different nutritional or physiological state and therefore may solve the same problem in a different way. This demonstrates the flexibility and robustness in social insect colonies during problem solving, e.g. see [120] and Ch.9 in [9]. Differences in the results also highlight the importance of conducting such an experiment on more than one colony; the conclusions that would be drawn if only one of the four colonies was used would be very different depending on which colony was chosen.

Similarities among the colonies are also illustrated by the increase in speed of internal workers, the use of rosettes during trophallaxis and crowding into the arena under the famine relief treatment. These are all features which are likely to contribute to faster transmission of food. One of the focal results of this thesis, presented in Chapter 4, is that under the famine relief treatment all four colonies consistently distribute food to around 90% of their workers within 30 minutes thereby efficiently relieving the famine, see figure 4-8. As the data from this experiment is explored in the next three chapters I will look for consistency, usually at the colony level, while also looking at various features in detail to highlight differences between the colonies.

Trophallactic exchanges occurring with multiple recipients is not a new result, as it has previously been reported in fire ants [79]. It will however influence how the net food each individual receives is calculated. When multiple recipients are receiving from one donor we must consider whether they are all receiving the same volume of food as a single recipient receiving from one donor. This problem is addressed in Chapter 6.

The change in behaviour between the two treatments is the focus of this project. Chapter 4 looks at how the rate of distribution of food is increased under the famine relief treatment in comparison to under control (Objective A). Chapter 5 focuses on the change in space use of the colonies between the two treatments (Objective B); the coverage of the brood pile by workers mentioned in this chapter is one aspect of this. Chapter 6 investigates the amounts of food individuals receive (Objective C) and the transmission pathways used between the workers under both treatments (Objective D).

Chapter 4

Food transmission from a temporal perspective

This chapter primarily concerns objective A: to compare the rates of feeding under the two treatments and determine how faster rates are achieved during famine relief. With this in mind most of the analysis in this chapter is from a temporal perspective (in comparison to Chapter 5 which is primarily from a spatial perspective). It is clear that we should expect an apparent difference in feeding activity between the two treatments: the ants are hungry under the famine relief treatment and therefore the transmission of food is likely to be a priority whereas under control there is less pressure to distribute food. When workers' energy stores are low time spent inactive will increase and eventually they will die leaving the brood and queen vulnerable [85]. It is therefore important to provide the internal workers with food as rapidly as possible after a period of starvation so we expect a higher rate of feeding unfed ants during the famine relief treatment. However, it is not apparent how long this higher rate of feeding is expected to last and whether feeding effort is constant during the 30 minutes analysed from the famine relief treatment, this will also be explored in this chapter.

The features considered in this chapter include: a comparison of the level of feeding activity (all feedings) as a function of time between treatments and its relation to the number of foragers inside the nest during the famine relief treatment; a comparison of the rate of increase in the proportion of fed ants (first feedings) between the two treatments and a discussion of a model that fits the

data; the pathways used to transmit this first batch of food to each ant and the use of 'rosettes' to facilitate higher rates of feeding during famine relief; and the progression of subsequent feeding as a function of time. Several of these features have recently been published, see [121]. We will see that there are similarities in food transmission at the colony level which is perhaps not surprising given that all four colonies have the same aim: to distribute food rapidly after starvation. However we will also see differences in the finer details of how each organises the distribution of food. This differences are likely to be a consequence of the demographic and geometric variation highlighted in Chapter 3 which will impose different constraints on each colony.

Throughout this chapter and subsequent chapters the units used for time will vary between minutes and seconds depending on which data set the analysis or graph is based on. In general analysis based on spatial data uses the time unit of a minute whereas the feeding data is to the second or alternatively averaged over a minute where stated.

4.1 Level of feeding activity inside the nest

One of the questions this chapter aims to address is whether the feeding effort or activity is constant throughout the famine relief treatment. In this section I explore the level of feeding activity in two ways: the number of receptions as a function of time to investigate the rate feeding events occur and the proportion of ants in a colony receiving in trophallaxis as a function of time to see if feeding effort is constant.

4.1.1 Cumulative number of receptions

It is reasonable to expect a higher frequency of receptions under the famine relief treatment given that the ants are hungrier compared with under the control treatment. However, it is not obvious whether the events will occur at a constant rate for the duration of the treatment. Each reception is taken as a pairwise event with a donor donating food to a recipient. When there are multiple recipients feeding simultaneously from one donor, as described in Chapter 3, each reception is considered separately and the time each recipient started receiving food from

the donor represents the start of the reception. Using the start times of each reception, figure 4-1 shows the cumulative number of receptions as a function of time under both treatments for each colony.

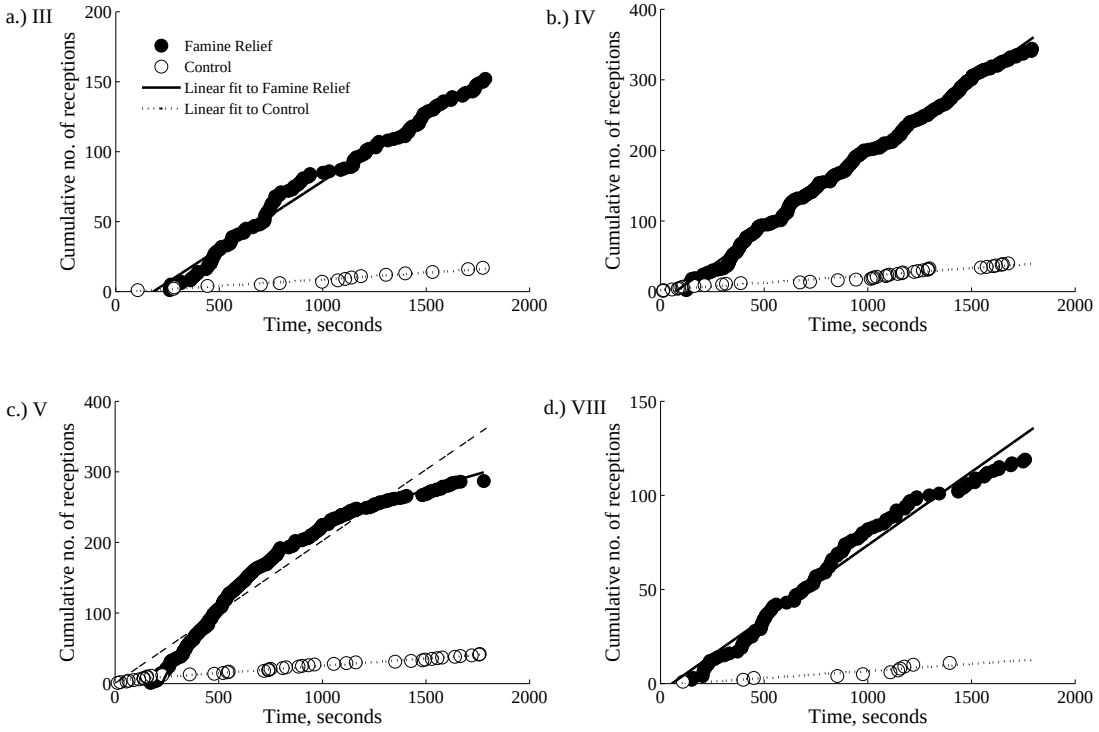

Figure 4-1: *Linear fits to the cumulative number of receptions against time. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77, dashed line represents linear fit, solid black line represent logarithmic fit; d.) VIII, N_c=49.*

The first point to notice from figure 4-1 is that, consistent with what we would expect, the number of receptions under the famine relief treatment is much larger than under control in all four colonies. Secondly, with the exception of colony V under the famine relief treatment, all treatments show a constant feeding effort over the 30 minutes, shown by a linear fit the details of which are presented in table 4.1. The R^2 values for the fits represent the proportion of the variability in the cumulative number of receptions that is accounted for by the statistical model and is calculated as the explained sum of squares over the total sum of squares [122].

The two larger colonies, IV and V, have comparable rate and number of receptions under the famine relief treatment and are higher than those of the two smaller colonies, III and VIII. This could also be anticipated as there are, by definition, more ants in the larger colonies so there are more workers contributing to the rate of interactions. However the average number of receptions per ant (total

number of receptions divided by the colony size) is roughly the same across all four colonies under famine relief (III=3.62, IV=3.62, V=3.73, VIII=2.48). This implies that at the densities used the colonies are not limited, in terms of the total number of interactions, by space availability. Previous work has shown that workers in *T.albipennis* allow approximately 5mm^2 per ant when constructing nest walls [92], the nests used in this experiment provide a much greater area per ant of between 13 and 30 mm^2. If much larger colonies had been used in the same size nests, for example 200 ants, the rates may not keep increasing with colony size if space availability becomes a limiting factor at such densities. Chapter 5 explores the effective density of each colony based on the area used by the workers.

It is apparent that the data for colony V in figure 4-1 under the famine relief treatment depart from the linear fit towards the end of the thirty minutes. In fact colony V is better fit by a logarithmic curve. This means the rate is fast at the start resembling the linear fit seen in the other three colonies, and then begins to slow down after approximately 15 minutes. This might happen if demand for food by the workers dies down as the colony becomes satiated. When the colony has reached satiation one might expect the rate of receptions to eventually drop to the level seen during the control treatment or lower if an excess of food was brought in during the famine relief process. To determine whether the rate continues to decrease would require the tracking of every worker inside the nest beyond the first 30 minutes of the famine relief treatment. For the reasons outlined in Chapter 2 this was not plausible given the methods available in the time of this project. A counter argument to the colony becoming satiated with food would be that the feeding activity decreases because lots of workers are leaving the nest to forage for themselves. In section 4.2 I explore whether this happens by looking at the number of workers inside the nest as a function of time bearing in mind that colony V already appears to be doing something different to the other three colonies. Colony V has a relatively small brood pile and larvae to worker ratio which are likely to allow more space inside the nest for feeding and a higher proportion of workers free to forage and distribute food compared with the other three colonies possibly resulting in colony V reaching satiation earlier.

The data for colony VIII also appear to depart from the linear fit towards the end, however the linear fit still had a higher R^2 value than the logarithmic. Perhaps this colony had started to reach satiation but not reached the same level as in colony V by the end of the treatment.

Treatment	Colony	Gradient	Sig.	R^2	Constant
Control	III	**0.010**	**0.000**	**0.971**	**-0.773**
	IV	**0.021**	**0.000**	**0.957**	**2.086**
	V	**0.021**	**0.000**	**0.982**	**4.526**
	VIII	**0.008**	**0.000**	**0.868**	**-0.844**
Famine Relief	III	**0.096**	**0.000**	**0.986**	**-17.53**
	IV	**0.209**	**0.000**	**0.996**	**-15.01**
	V	**0.202**	**0.000**	**0.927**	**0.538**
	V*	**145.2**	**0.000**	**0.989**	**-787.4**
	VIII	**0.078**	**0.000**	**0.973**	**-4.263**

Table 4.1: *Results from the regression analysis with cumulative number of receptions as the dependent and time as the independent variables. V* is the logarithmic fit. Bold entries indicate significant results.*

This initial analysis has shown that the feeding effort is constant, however it is not possible to deduce from it whether the organisation of feeding is constant, for example via purely pairwise events or feeding multiple recipients simultaneously in rosettes. I will explore this aspect later in this chapter.

4.1.2 Proportion of ants receiving food as a function of time

The second aspect of the level of feeding activity that I am going to explore temporally is the proportion of ants that are receiving food in trophallaxis. Before looking at this aspect as a function of time it is first instructive to have an idea of what the average and range of this proportion is for each colony under the two treatments.

Figure 4-2 shows the median proportion of ants in each colony, with interquartile range, that are recipients in trophallaxis at any one timestep over the 30 minutes of each treatment. The graphs in figure 4-1 were based purely on the start time of each reception; in contrast the representation investigated in this section is based on the duration of receptions. The figure again demonstrates the higher level of feeding activity under the famine relief treatment as the grey bars are all above the white bars representing the control treatment.

Colony VIII has the highest median proportion of ants receiving over the 30 minutes of the famine relief treatment. This is an interesting result when compared

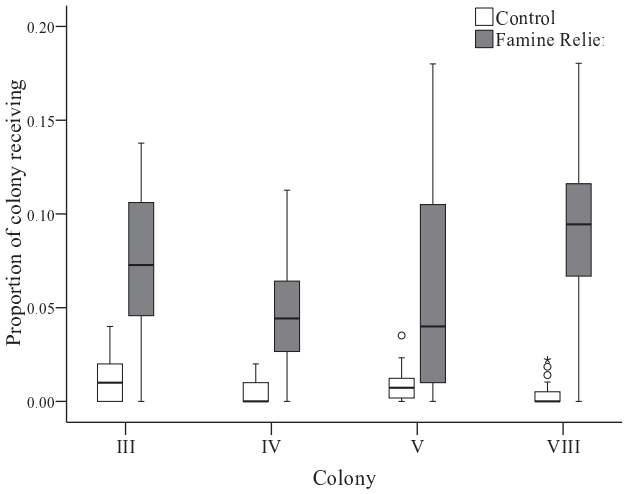

Figure 4-2: *Proportion of each colony receiving in a trophallaxis event at any one time during the 30 minutes of each treatment.*

to the average number of receptions per ant shown previously which was the lowest out of the four colonies. The combination of these two features imply that in colony VIII receptions tend to be slightly less frequent but longer in duration compared with the other three colonies. In Chapter 6 I explore the amounts of food distributed to individual workers based on the duration of receptions and will see if the four colonies bring in similar amounts of food per capita.

Colony IV has a low median proportion of ants receiving and a narrow inter-quartile range under the famine relief treatment. This could be explained by the colony being relatively less hungry than the others: perhaps this colony stored more food in workers' crops prior to the starvation period, see section 4.4.1, this possibility will be explored in Chapter 6. It appears that this colony may be able to achieve a comparable number of receptions by making the durations shorter resulting in a lower overall level of receiving compared with the other colonies. This compromise in colony IV may arise due to the limitation on space available for feeding due to the large brood pile.

Colony V has a noticeably wide variability in the proportion of ants receiving shown by the inter-quartile range in figure 4-2. We know from figure 4-1 that the rate of receptions slows down in the second 15 minutes under famine relief. Looking at the feeding activity as a function of time will reveal whether there is a temporal pattern in this variation. One might expect there to be a sharp rise in activity at the start of the famine relief treatment in all colonies as the food

is first brought in initiating the switch from the starvation period to distributing food for famine relief.

Figures 4-3 and 4-4 show the proportion of ants receiving as a function of time for each colony under the control and famine relief treatments respectively. All colonies under famine relief show the expected sharp rise in food receiving activity at the start as they change abruptly from a starvation period to a period of famine relief. These figures demonstrate that for three of the colonies, III, IV and VIII, under the famine relief treatment the level of food receiving activity as a function of time can be described as constant with large fluctuations. In fact the fluctuations are not so large in colony IV and I will explore why this is in the next section. In contrast, colony V shows a large peak in food receiving activity within the first 10 minutes and then begins to decline for the remaining 20 minutes. This behaviour explains the large variability seen for colony V's overall level of food receiving activity seen in figure 4-2. By the end of the famine relief treatment colony V has a very low level of food receiving activity which corresponds to the decline in rate of increase in the cumulative number of feeding events seen in figure 4-1. We might expect the food receiving activity to peak when foragers are inside the nest. I now explore whether the fluctuations in food receiving activity in colonies III, IV and VIII and the sharp rise and then decline in food receiving activity in colony V can be explained by the number of ants inside the nest as a function of time as a proxy for when foragers are inside the nest.

4.2 Proportion of ants inside the nest as a function of time

The proportion of tracked ants inside the nest as a function of time can be deduced from the spatial point samples of all the ants in each colony. At each minute every individual is recorded either at a location inside the nest, or simply as outside the nest. Again it is informative to know the overall variability in the proportion of ants inside the nest accumulated for the 30 minutes for each colony under the two treatments before we look at it as a function of time. This will show whether there are overall differences between colonies and between treatments. The number or proportion of workers inside the nest may vary between colonies

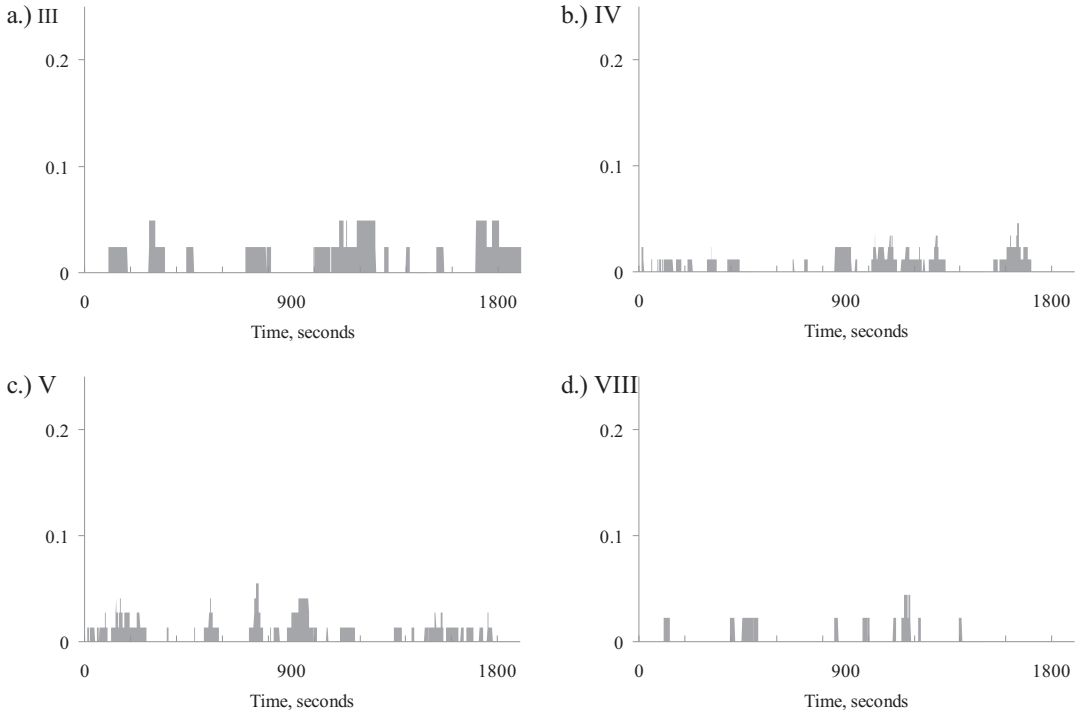

Figure 4-3: *Food receiving activity inside the nest as a function of time under the control condition.* $P_{receiving}$ *represents the proportion of the colony participating in a trophallaxis event as a receiver. The scale of* $P_{receiving}$ *is chosen to allow comparison with figure 4-4, a.) Colony III,* N_c*=42; b.) IV,* N_c*=95; c.) V,* N_c*=77; d.) VIII,* N_c*=49.*

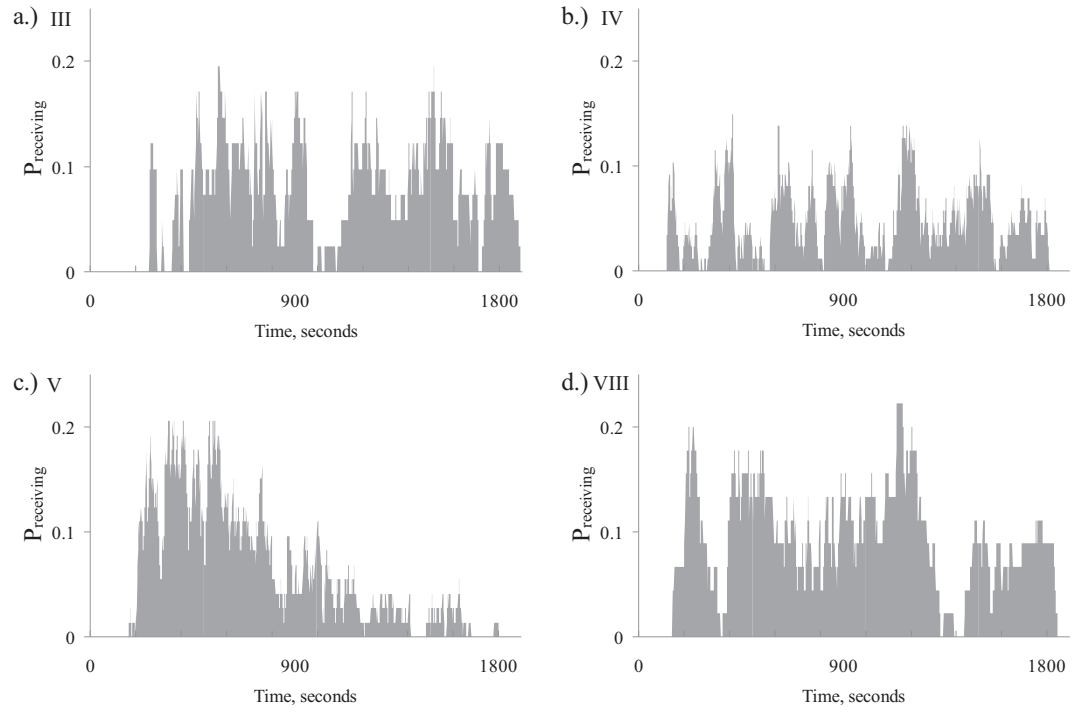

Figure 4-4: *Food receiving activity inside the nest as a function of time under the famine relief treatment.* $P_{receiving}$ *represents the proportion of the colony participating in a trophallaxis event as a receiver. a.) Colony III,* N_c*=42; b.) IV,* N_c*=95; c.) V,* N_c*=77; d.) VIII,* N_c*=49.*

particularly as there is, for example, variation in ratio of brood items to workers, see figure 3-2, which may influence how many workers are free to leave the nest. Figure 4-5 shows the proportion of tracked ants in the nest averaged over the 30 minutes for each treatment. It is apparent that in all four colonies there are more ants inside the nest under the famine relief treatment.

The fact that more ants are inside the nest during the famine relief treatment is in itself an interesting result and is not explained by the relatively small differences between treatments in the number of ants tracked in each colony as presented in table 2.2. One might imagine that the optimal strategy to adopt when food is being brought into the nest would be to recruit as many workers as possible to leave the nest and help bring in the food. It appears however that the colonies are not adopting this strategy and several of the potential foragers are remaining inside the nest. A similar result was observed in *Leptothorax acervorum* where more workers were found inside the nest once food had been re-introduced after starvation [88]. The authors suggest the high level of workers inside the nest was to allow the distribution and digestion of food. Later chapters will explore why in this study the colonies are choosing this strategy and what role the ants who remain inside the nest play.

Figure 4-5 also shows that colony V has many fewer ants inside the nest under both treatments and a wider inter-quartile range than the other three colonies. In comparison colony IV has the smallest inter-quartile range. Again, these differences may be related to the demographic differences between the colonies. Colony V has a relatively small brood pile and few larvae and therefore can perhaps invest more workers in external tasks such as foraging. Colony IV on the other hand has a very large brood pile which would require a large proportion of the workers to maintain and therefore potentially not as many workers are available to leave the nest.

Now that we have a picture of the average and variation of the proportion of tracked ants inside the nest for each colony the proportion inside the nest as a function of time can be investigated as shown in figure 4-6. The figure again highlights that under the famine relief treatment the proportion of workers inside the nest is nearly always higher than that under control, with the exception of a small period at the start for colony V.

To determine whether there was a trend for the proportion inside the nest as a function of time I performed a linear regression analysis on each of the data sets.

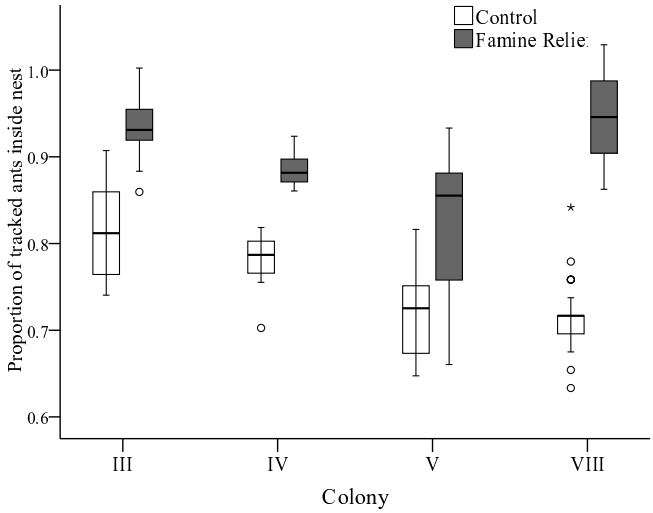

Figure 4-5: *Proportion of tracked ants inside the nest averaged over the 30 minutes of each treatment.*

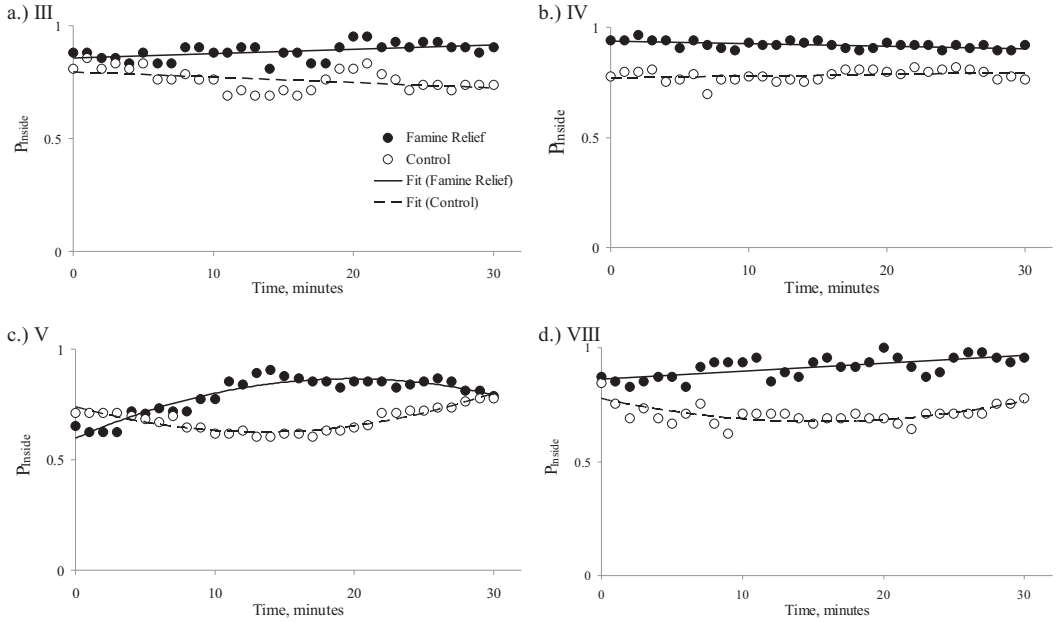

Figure 4-6: *Proportion of tracked ants inside the nest as a function of time. Lines represent linear fits to the data with the exception of colony V both treatments and colony VIII for the control treatment where quadratic fits are used, see table A.1. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

Table 4.2 contains the results of this analysis. It shows that in all colonies there is a small change in the proportion with time. The data for colony V famine relief treatment and control and colony VIII control are better fitted by a quadratic, see table A.1 in appendix A. Figure 4-6 shows these fits to the data. It is

noticeable that colonies III and VIII have some fluctuations away from the linear fit, particularly under famine relief, whereas colony IV has much less variation as previously noted from the inter-quartile range in figure 4-5. These differences are likely to be due to the differences in colony size, the smaller colonies being more susceptible to noise due to small N_c.

Treatment	Colony	R^2	F	Sig.	Constant	Gradient
Control	III	**0.199**	**7.188**	**0.012**	**0.796**	**-0.002**
	IV	0.094	2.998	0.094	0.768	0.001
	V	0.121	3.989	0.055	0.646	0.002
	VIII	0.000	0.009	0.927	0.710	0.000
Famine Relief	III	**0.249**	**9.610**	**0.004**	**0.857**	**0.002**
	IV	**0.342**	**15.05**	**0.001**	**0.939**	**-0.001**
	V	**0.513**	**30.61**	**<0.005**	**0.691**	**0.007**
	VIII	**0.438**	**22.64**	**<0.005**	**0.860**	**0.003**

Table 4.2: *Linear regression on the proportion of tracked ants inside the nest over time. Bold entries indicate significant results.*

4.2.1 Correlation between ants inside the nest and level of food receiving activity

As seen in figure 4-4 the level of food receiving activity as a function of time, represented by the proportion of ants receiving at each time-step, can be described as constant with fluctuations in three out of four of the colonies. The proportion of tracked ants inside the nest also fluctuates. I will now explore whether the fluctuations in the proportion of tracked workers inside the nest are correlated with the level of food receiving activity. A higher proportion of workers inside the nest would suggest that foraging individuals have returned to the nest with food which in turn would lead to a higher level of feeding activity.

The spatial data were collected every 60 seconds; in contrast the feeding data were collected to the resolution of one second. To compare the two, I have taken an average of how many ants are feeding over 60 second periods that correspond with the times of the spatial point samples. For example, to correspond with the spatial point samples collected at $t = 60$ seconds, I took an average of the proportion of workers receiving for the period $30 \leqslant t < 90$ seconds.

A Pearson's correlation test on these data sets shows that in colonies III and VIII the food receiving activity is positively correlated with the proportion of

tracked ants inside the nest, see table 4.3. Colonies IV and V did not show a significant correlation between food receiving activity and the proportion of ants inside the nest. As colony IV shows little variation in either it is unlikely that a strong correlation will exist between the proportion of ants inside the nest and the level of food receiving activity. However to look for a pattern by eye, I have plotted both the proportion of tracked ants inside the nest and the level of food receiving activity on the same graph in figure 4-7. This figure highlights nicely that in colonies III and VIII the peaks and troughs in the proportion of ants inside the nest coincide with the peaks and troughs in food receiving activity. There is a tendency for this to happen in colony IV also but the troughs in the food receiving activity, seen between $t = 5$ to $t = 10$ minutes and between $t = 15$ and $t = 20$ minutes, are more pronounced compared with the more gradual decline in the proportion of ants inside the nest at these times. This is likely to be because the proportion of ants inside the nest for colony IV shows little variation in comparison to the other three colonies.

Colony V appears to be a completely different case from the other three colonies. There is a large peak in food receiving activity in the famine relief treatment which begins to die down after the first ten minutes. The proportion of ants inside the nest however increases during the first 15 minutes then appears to level off. It would seem as though this colony is very active during the first ten minutes in terms of feeding. As the colony starts the treatment with a relatively low proportion of workers inside the nest, we can speculate that there were more workers foraging at $t = 0$ seconds compared to the other three colonies. This would then lead to a higher number of foragers returning to the nest to carry out the feeding inside the nest in the ten minute period at the start of the famine relief treatment. Then perhaps, as the colony managed to perform the feeding so efficiently early on, the feeding dies down and the foragers remain inside the nest as the colony has become satiated. In the following section I will look at how efficient the colonies are at relieving the famine and probe further into why colony V appears to be different.

4.3 First feeding events

A reasonable measure of colony efficiency in famine relief is the rate with which food reaches the unfed members. Feeding events which involve the transfer of

			Sig. for K-S test for Normality	
Colony	**Pearson correlation coefficient**	**Sig.**	**Spatial**	**Feeding**
III	**0.664**	**0.000**	0.251	0.752
IV	0.073	0.695	0.188	0.992
V	−0.065	0.729	0.181	0.329
VIII	**0.529**	**0.002**	0.371	0.931

Table 4.3: *Correlation results for the proportion of tracked ants inside the nest with the level of food receiving activity. Bold entries indicate significant correlations. All data sets were not significantly different from normal, tested using Kolmogorov-Smirnov normality test.*

food to an unfed ant are 'first feeding events'. By definition each ant can only be a recipient of a first feeding event once; therefore by looking at the cumulative proportion of ants which become fed as a function of time we can investigate the rate of first feedings, i.e. the efficiency of the famine relief process. During the control treatment we do not expect this rate to be particularly high because the workers are not starved. We also already know from figure 4-1 that the overall rate of feeding is relatively low in comparison to that under the famine relief treatment. Under the famine relief treatment we might expect the colonies to transmit food to unfed ants at a much higher rate given that the workers have endured a starvation period and there is a high level of overall feeding activity.

We define all ants as 'unfed' at the start of each treatment. Prior to the famine relief treatment the colonies are deprived of food for two days. Therefore at the start of the treatment the ants are truly unfed as they cannot yet have received any of the newly provided honey solution. Food has been provided regularly prior to the control treatment so, unlike the start of the famine relief treatment, in the control the starting point is somewhat arbitrary in terms of the nutritional state of the colony before and after. However, to make a comparison we are only interested in how many ants become fed within an equal duration to the famine relief treatment, i.e. 30 minutes beginning at the start of the treatment. Therefore the ants are also considered 'unfed' at the start of the control.

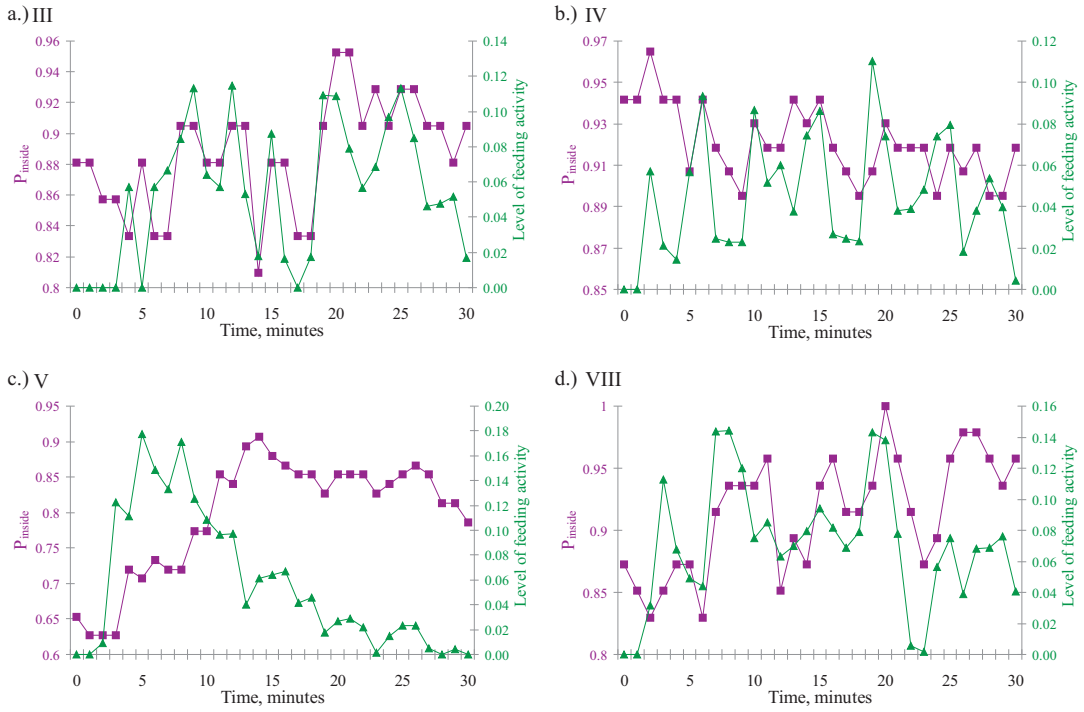

Figure 4-7: *Proportion of tracked ants inside the nest and level of food receiving activity against time. Purple squares represent the proportion of tracked ants inside the nest, green triangles represent level of food receiving activity. Lines between points to guide the eye. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

4.4 Determining when each worker becomes fed for the first time

I designed an algorithm to search the trophallaxis data and identify the first feeding event for each ant. In this algorithm there are two ways in which ants may switch from unfed to fed; once fed an ant cannot switch back to being unfed during a single treatment. Firstly, ants may become fed for the first time by receiving food from another ant during a trophallactic exchange inside the nest. This may be from a forager or from another internal nest mate who has food available to donate. Secondly, if an individual participates in a feeding event as a donor before they are involved in any other feeding event, we assume that they had food to donate prior to this event and therefore consider this event as their 'first feeding event'. An individual may have food available to donate in this way via two means: firstly, if the individual is a forager, i.e. they leave the nest and drink at the honey solution directly; and secondly, if they had food stored in their crop prior to the start of the treatment. This is a special case which I have

called 'background feeding' and will be explained and explored in more detail in Chapter 6.

Although information about which individuals drank from the honey solution was recorded on the audio channel, it was not an exhaustive data-set as explained in Chapter 2, see section 2.3.4. Therefore the criterion used to determine whether an ant drank from the honey solution is that they must have spent a period of time outside the nest (determined using the spatial point samples) and subsequently have donated food to another ant inside the nest. There are a small number of ants in each colony (between 1 and 4) that are recorded on the AC as drinking from the honey solution and then never subsequently donated. There could also be some ants that spend time outside the nest and never subsequently donated to a nest mate. One could argue that these workers also became fed because they had the opportunity to drink at the honey solution. However, there is no evidence the second type ever located the honey solution, in which case we would be over-estimating the number of fed ants. While the ants that did drink at the honey and did not subsequently donate did not contribute to the distribution of food within the nest. Using the criterion above gives a conservative estimate of the number of ants that are fed through being a forager and is consistent between the two treatments.

The time used to represent when each individual switches from unfed to fed, is the start time of the first feeding event they participate in, regardless of whether they are a donor or recipient in that event. This is used even for individuals which are foragers because as just mentioned the data for individuals drinking at the honey are incomplete so there is not a specific start time for every drinking event. This subset of the feeding events that contain the first feedings for each individual are from now on referred to as the 'first feeding data'.

4.4.1 Truncating the first feeding data

There is a need to truncate a small number of events from the start of the first feeding data for the famine relief treatment. In Chapter 2 section 2.2, I described how filming of the treatments began when the first ant that finished drinking at the honey solution started to make its way back to the nest, i.e. the first laden forager returns. This means there is a delay in time between when the films began, T1, and the time that the first forager entered the nest, T2, and

subsequently when a laden forager donated food for the first time inside the nest, T3. Table 4.4 shows these times for each colony.

From the trophallaxis data it was clear that under the famine relief treatment, in three out of four of the colonies, several feeding events occurred before the first donation by a forager, T3. Table 4.4 gives the number of these feeding events which occur before T3, the start time of the first of these events is represented as T4. In the three colonies where T4 $<$ T3 feeding events which started between T4 and T3 were truncated from the first feeding data. These events represent the transmission of food that has not originated from the newly supplied honey solution as they occur before the new food has entered the nest via a returning laden forager. The food must therefore have been stored in the crops of the workers during the starvation period. This suggests that some workers had retained food from during the control treatment, the last possible time during which they could have stored food. Feeding events of this nature will be referred to as 'background feeding'.

Background feeding may also occur after the start of the transmission of the new food, t =T3, if some ants still contained stored food. These events can still be considered in the first feeding data, if they are the first event for an ant, as after T3 the state of the colony changes from starvation to famine relief. These events are different from background feeding events that occur before T3 which are not part of the famine relief process but can be considered as feedings to stave off starvation until famine relief commences with the return of a laden forager.

Time T5 in table 4.4 gives the time that the first forager to donate returned to the nest. (Note the ants returning at time T2 did not always subsequently donate and therefore might have been unsuccessful foragers, i.e. did not drink at the honey solution.) The small delay between the time these laden foragers entered, T5, and the start time of their first donations, T3, gives an indication of the speed with which the feedings occurred under the famine relief treatment. In colony VIII the first feeding event to occur was from the first laden forager that returned at $t = 147$ seconds (i.e. T2=T5 and T3=T4 in this case), therefore no events were truncated from this colony. Three events were truncated from each of colonies III and V and 6 events from colony IV, these are relatively small in comparison to the total number of reception events in each colony (III=155, IV=350, V=290, VIII=119). Ants which were involved in the truncated events were included in the first feeding data if they were involved in another feeding event after T3.

Colony	**III**	**IV**	**V**	**VIII**
T1	0	0	0	0
T2	2	35	145	147
T3	261	124	171	150
T4	116	1	26	150
T5	249	116	156	147
Number of events truncated	3	6	3	0

Table 4.4: *Times used to determine when the first feeding data should be truncated, unit is seconds: T1 - time video started, T2 - time the first forager returned to the nest, T3 - time of the first donation by a returned, laden forager occurred, T4 - time of the first feeding event observed on the video inside the nest, T5 - time that the first laden forager to donate returned to the nest. Where T4 < T3 feeding events between T4 and T3 were truncated.*

The first feeding events under the control treatment do not need to be truncated as the return of the first laden forager after the start of the video, T1, does not trigger a change in state of the colony. Background feeding events that happen before T3 under control are considered to be the same as those which occur after T3. Therefore all the first feeding events from the start of the video, T1, were included for the control treatment.

4.5 First feeding curves

The cumulated proportion of ants that become fed as a function of time is a measure of efficiency during famine relief and is shown in figure 4-8 where all members of each colony are classed as unfed at the start of both treatments, time $t = 0$ seconds. These curves will be referred to as the 'first feeding curves'. The upper limit for the cumulative proportion fed, K, is taken as the total number of ants fed under the famine relief treatment, see table 4.5. This number was chosen as it would appear that this is the limit that 'want' to be fed. This K is analogous with the "colony-desired harvested volume", K, in [78].

Figure 4-8 shows that by the end of the thirty minutes of the famine relief treatment around 95% of the ants in each colony had received food in comparison to around 40% of each colony during the control treatment. This shows, as expected, that under famine relief a lot more of the ants become fed compared to under control. Table 4.5 gives the actual numbers of workers fed in the two treatments and the proportion of fed workers whose first feeding event was as a

Colony	**No. in colony,** N_C	**No. fed in CC**	**No. fed in FR,** (K)	$\mathbf{P_{FFD}}$ **in CC**	$\mathbf{P_{FFR}}$ **in CC**	$\mathbf{P_{FFD}}$ **in FR**	$\mathbf{P_{FFR}}$ **in FR**
III	42	18	41	0.22	0.78	0.17	0.83
IV	95	47	87	0.40	0.60	0.24	0.76
V	77	47	73	0.55	0.45	0.40	0.60
VIII	48	17	45	0.47	0.53	0.20	0.80

Table 4.5: *Number of ants fed in each colony during both treatments.* P_{FFD} *is the proportion of fed ants that are first fed by being a donor,* P_{FFR} *is the proportion of fed ants that are first fed by being a recipient.*

donor or a recipient. In colony V under famine relief a larger proportion of ants' first feeding event was as a donor compared to the other three colonies. At this point we cannot discern from the first feeding curves whether these donors were foragers or ants involved in background feeding, this will be addressed in section 4.8. However, the large proportion of ants first fed as donors under the famine relief treatment may explain the high level of food receiving activity during the first ten minutes in colony V, seen in figure 4-4 and also why this colony reaches K in roughly half the time of the other three colonies.

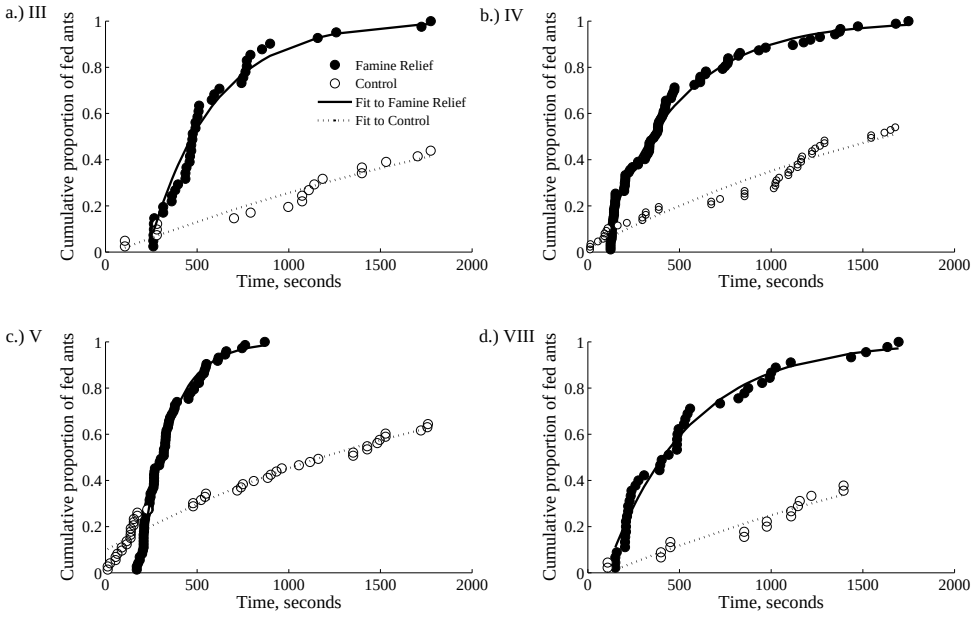

Figure 4-8: *First Feeding Curves: Cumulative proportion fed against time. Lines represent recovery exponential fits. a.) Colony III,* N_c*=42; b.) IV,* N_c*=95; c.) V,* N_c*=77; d.) VIII,* N_c*=49.*

It is clear from figure 4-8 that all four colonies are able to relieve the famine efficiently in comparison to the rate of feeding under normal conditions and colony V does so much quicker than the other three. The first feeding data under control appear to follow a relatively gentle linear slope. In contrast under famine relief the first feeding data in all four colonies have an initial very steep increase followed by a leveling off. Given that there are demographic and geometric differences between the colonies and already in some aspects of the feeding behaviour, it is perhaps remarkable that all four colonies show this same pattern.

4.6 Curve fitting

Given that all four colonies express the same pattern in the first feeding curves it is appropriate to devise a mathematical model which describes this aspect of the famine relief process. To aid the process of developing such a model several equations and simple functions were tested to see if they fit the shape of the first feeding curves. Candidates for the best fit were selected based on: their simplicity; having solutions with a shape that resembles that of the first feeding curves, i.e. convex from above; and how they can be interpreted in a biological context, i.e. the process of distributing food among a system of feeders and unfed ants which become fed. The equations tested included the logarithmic growth, recovery exponential and Michaelis-Menten equations. Figure 4-9 shows an example of these three curves for the purpose of demonstration and table 4.6 gives the equations and parameters used in figure 4-9. To make the curves comparable I have set $t(50) = 100$ seconds. and initial number fed, N_0, to equal 1 where applicable.

Because the first feeding curves are convex from above we can rule out sigmoid functions such as the logistic, however we will later see how the biological interpretation of the logistic equation in the context of a distribution process is related to the model that best fits the data. The other functions tested include the inverse and square-root t functions; these are included as other simple functions which give a curve which is convex from above mostly for completeness as it is unlikely that there is a simple distribution model that describes such functions. Clearly there are alternative equations beyond the three tested that could fit the data better, however the three equations were chosen for their simplicity which is desirable when developing a model. In addition, K is discrete and relatively

small when compared to the size of systems which can be described by more complicated models. These features mean that the amount of noise in the first feeding curves, particularly once they enter the negative-acceleration phase, is too large to justify using a more complex model.

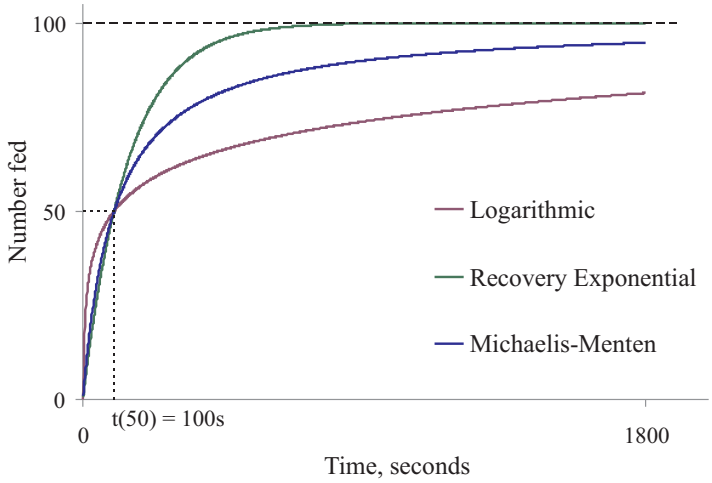

Figure 4-9: *Examples of logarithmic, recovery exponential and Michaelis-Menten fits to first feeding curves for number fed, N, against time, t. For these examples K is set to 100, N_0 to 1 where relevant, and $t(50) = 100$ seconds.*

It is clear from figure 4-9 that although all three curves reach $N = K/2$ by 100 seconds, the shapes are different. In the context of the ants the shape of these curves will have different implications for how the food distribution process progresses as a function of time. These differences can be demonstrated by looking at processes that each of the tested equations are used to describe.

Logarithmic growth is seen in microbiology during the rapidly growing exponential growth phase of a cell culture, e.g. [123]. The exponential distribution occurs naturally when describing lengths of inter-arrival times in a homogeneous Poisson process, for example predator-prey encounter rates in mathematical models when the distribution of prey is spatially patchy, see [124]. The corresponding exponential cumulative distribution function or the recovery exponential has been used to describe the quantity of food inside the nest of a *Formica fusca* colony [78]. The basic form of the Michaelis-Menton equation is;

$$v_0 = \frac{V_{max}}{1 + \frac{K_M}{S}}, \tag{4.1}$$

Equation	**Solution**	***Value of shape parameter** $a - c$	**Form used for curve fitting**
Logarithmic	$N = a \ln t$	10.86	$n = C + a \ln t$
Recovery exponential	$N = K(1 - \exp(-bt))$	0.007	$\ln(1 - n) = C - bt$
Michaelis-Menten	$N = K/(1 + 1/ct)$	0.01	$\ln(1/n) = C + (1/ct)$

Table 4.6: *Formulae for the equations tested. Where applicable the parameters are: K, the upper asymptote; N_0, the y intercept; and the additional shape parameter is labeled $a - c$ for each progressive equation. * Values for example curves shown in figure 4-9, where $K = 100$, $t(50) = 100$ seconds, and $N_0 = 1$ where applicable. The forms of the equations shown in the last column are used during the curve fitting and use proportions, n, as opposed to numbers, N, of fed ants.*

where v_0 is the reaction rate, V_{max} is the maximum rate of the reaction, i.e. the asymptote, K_M is the Michaelis constant and S is the concentration of the substrate. It is used in the kinetics of many enzymes and describes the rates of irreversible reactions [125]. The feeding of an ant can also be viewed as an irreversible transition from an unfed state to a fed state. The Michaelis-Menton equation is used in situations where the kinetics are very simple; the concentration of the enzyme, E, and intermediate complex do not change as a function of time (so are not included in equation 4.1). The enzyme concentration, E, is synonymous with the number of ants acting as feeders to unfed ants and the substrate, S, with the number of unfed ants, $K - N$. The reaction rate v_0 corresponds to the number of fed ants, N, while the maximum rate V_{max} corresponds to the upper limit of ants that will be fed, K.

To test the fit of the first feeding data to these three equations, a linear regression was performed on the data for the forms of each of the equations shown in table 4.6. Of the equations tested, the recovery exponential gave the best fit to the growth of the proportion of newly fed ants as a function of time in all four colonies. The results for the fit of the recovery exponential are shown in tables 4.7 and 4.8, the details of the fits for the logarithmic and Michaelis-Menten equations and inverse and square-root functions can be found in Appendix A. The R^2 values for the recovery exponential fits are higher than for the other equations tested for each data set. The recovery exponential also fits the data from the control; this will enable a comparison of the rates between the two treatments within colony as well as between colony, see figure 4-12.

To further verify that the recovery exponential was the best fit to the data, an Anderson-Darling test for normality was performed on the standardised residuals from the fits of all three equations (and the two functions) [126]. The results from the Anderson-Darling test for all four colonies are shown for the recovery exponential in table 4.7 and figures 4-10 and 4-11 and for the other candidates tested in Appendix A. Table 4.7 shows that for the recovery exponential for 4 out of 8 of the data sets the standardised residuals were normally distributed and one near-normally distributed (method should be robust to near-normality). In comparison for the logarithmic 6 out of 8 and for the Michaelis-Menton 4 out of 8 data sets were normally distributed or near-normally distributed. The inverse and square-root functions also did not show a better fit compared with the recovery exponential based on the R^2 values and normality tests. In combination with the highest R^2 values this means that the recovery exponential was the best

fit overall out of the three equations (and two extra functions) tested. Again it is important to emphasise that such a consistent result across all four colonies is remarkable given the differences between them that we have noted to this point.

Treatment	**Colony**	**Mean**	**Standard deviation**	**N**	**A-D**	**Sig. for A-D normality test for residuals**
Control	III	−0.01956	1.019	18	0.550	0.134∗
	IV	−0.00546	1.007	47	1.099	0.006
	V	0.00089	1.012	47	0.371	0.409∗
	VIII	−0.02092	1.028	17	0.249	0.704∗
Famine Relief	III	0.01942	1.082	41	0.890	0.021∗
	IV	−0.00042	1.027	87	1.966	< 0.005
	V	−0.00536	1.034	73	3.708	< 0.005
	VIII	−0.00271	1.039	45	0.385	0.378∗

Table 4.7: *Detailed results of Anderson-Darling test for normality on standardised residuals from the recovery exponential fit to the first feeding data. N is the number fed. * Indicates data set is normal or near normal.*

Table 4.8 shows the rates of the recovery exponential fits to the first feeding curves. As expected due to the difference in nutritional state of the colonies between the two treatments under famine relief the rate is an order of magnitude greater than under the control treatment for all four colonies. This is also shown in figure 4-12 which highlights that the two larger colonies, IV and V, show slightly faster rates in the control treatment than the two smaller colonies, III and VIII, which have comparable rates. This may be an example of an economy of scale where the larger colonies are more efficient at distributing food. Note that the overall rate of feeding taken from figure 4-1, not just first feeding, is also higher in the larger colonies (Average number of receptions per ant under control: III=0.33, IV=0.42, V=0.55, VIII=0.23).

Comparing the rates for the famine relief treatment, colony V has the fastest rate which implies that this colony is the most efficient in relieving the famine in terms of distributing food to unfed individuals inside the nest. As I mentioned earlier in this chapter, colony V appears to employ a different strategy for relieving the famine. A much lower proportion of workers are inside the nest at the start of the treatment, ($P_{Inside}(0) = 0.65$) in comparison with the other three colonies at the start of the famine relief treatment ($P_{Inside}(0) = 0.87$ to 0.94), see figure 4-6. This suggests the colony has a higher proportion of foragers which is likely to contribute to a higher rate of feeding. A higher proportion of foragers increases

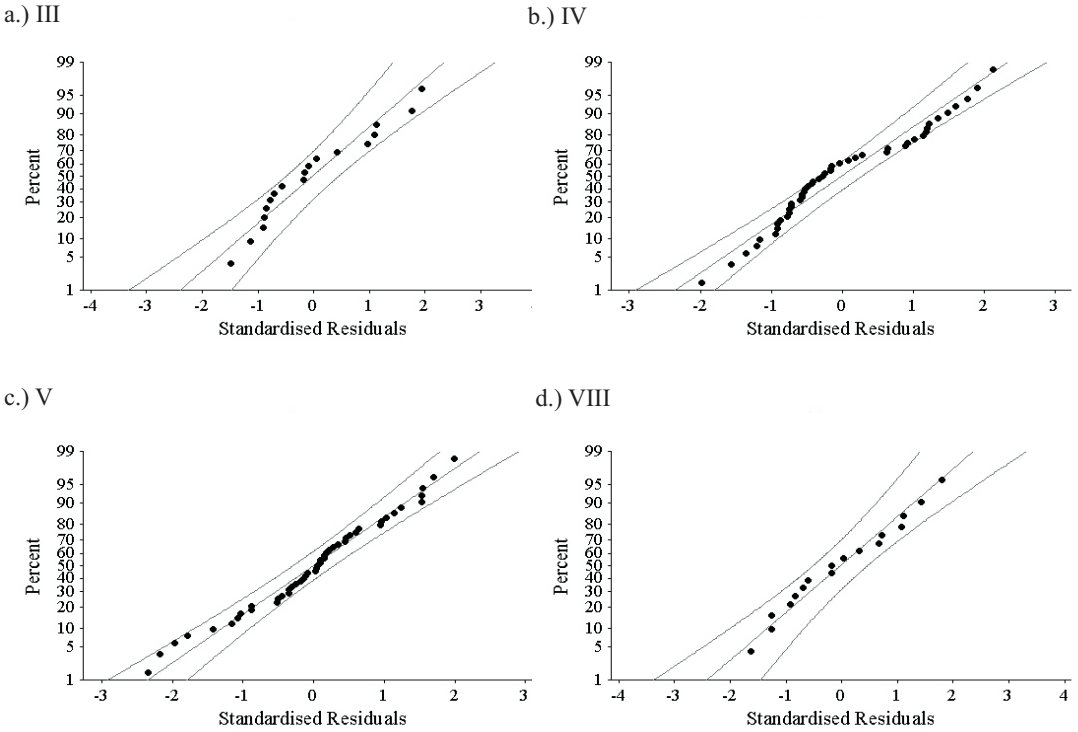

Figure 4-10: *Probability plots of standardised residuals from recovery exponential fit to first feeding under the control treatment. Testing for normal distribution with 95% Confidence intervals. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

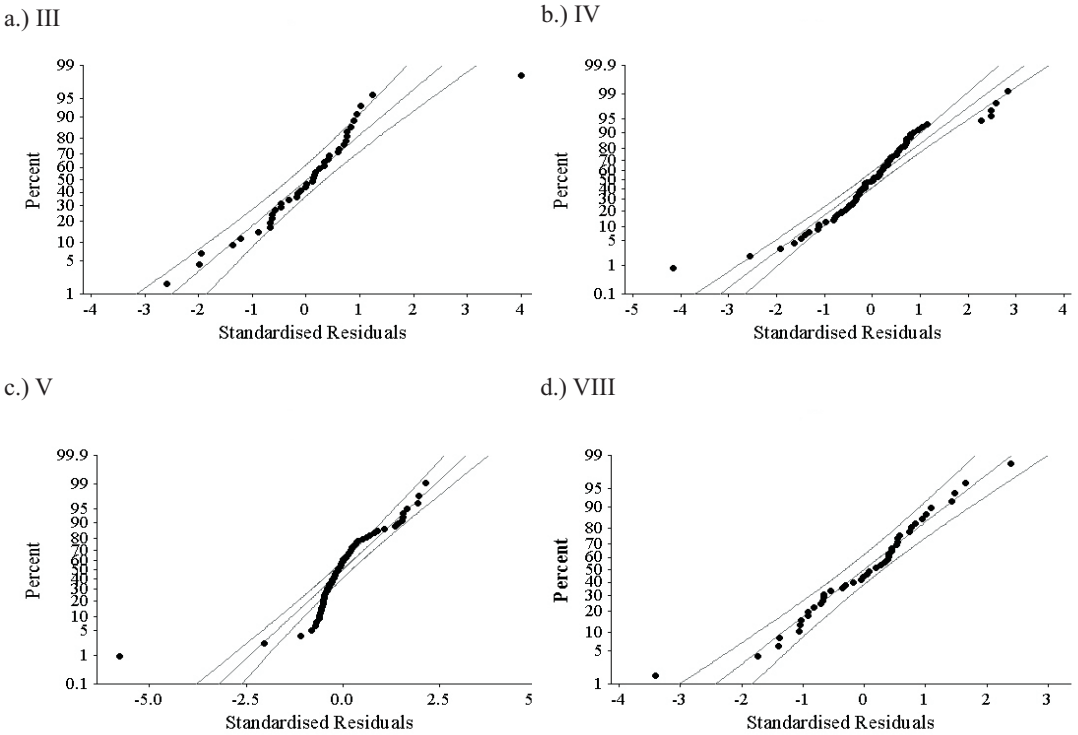

Figure 4-11: *Probability plots of standardised residuals from recovery exponential fit to first feeding under the famine relief treatment. Testing for normal distribution with 95% Confidence intervals. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

the rate in two ways: there are more foragers to feed unfed ants but also the foragers themselves are classed as fed once they donate. This second effect is seen by the higher proportion of the first feeding events for ants in colony V that are as donors, see table 4.5.

Treatment	**Colony**	**Gradient**	**SE for gradient**	$\mathbf{R^2}$
Control	III	0.00031	0.000019	94.6
	IV	0.00041	0.000015	94.3
	V	0.00050	0.000012	97.4
	VIII	0.00032	0.000024	92.3
Famine Relief	III	0.0028	0.00009	96.3
	IV	0.0025	0.00004	98.1
	V	0.0062	0.00011	98.0
	VIII	0.0022	0.00005	97.9

Table 4.8: *Gradients of recovery exponential fits to first feeding curves*

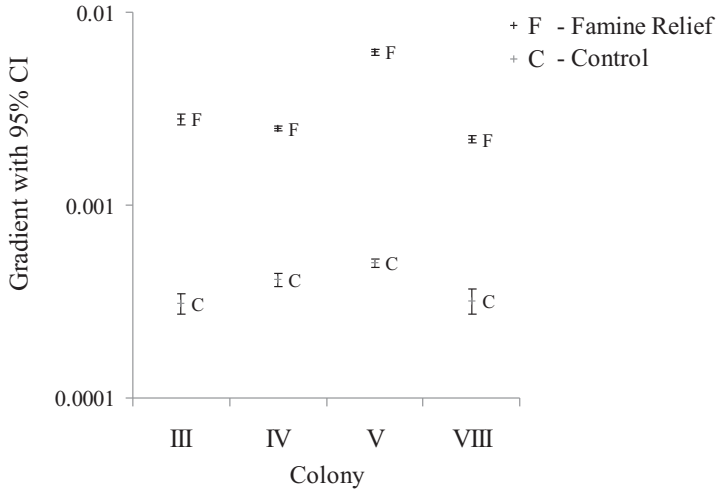

Figure 4-12: *Sample estimates of the gradient in the fitted recovery exponential model with 95% confidence intervals.*

4.7 Model derivation

Now it has been shown that of the equations tested the recovery exponential equation best describes the first feeding curves the next step is to use this knowledge to interpret the famine relief process in terms of a mathematical model. For convenience in such a model we can assume that the number of fed ants, N, and time, t, are continuous variables. Each colony can be regarded as a small

discrete system as each colony is less than 100 individuals and in the context of this experiment is closed, i.e. assume there are no births, deaths or emigrations during the treatments. Continuous models are widely and successfully used for small discrete systems, for example to model tumor growth or in biochemical kinetics [127]. Therefore we expect the model to be an ordinary differential equation representing the increase in the number of fed ants, $\mathrm{d}N$, for a small change in time, $\mathrm{d}t$. Imagine there are a number of ants working as 'feeders' who when they encounter an unfed ant give them food (they also feed fed ants when they encounter them but these interactions do not contribute to the increase in N). Then the increase in the number of fed ants, $\mathrm{d}N$, in a small amount of time, $\mathrm{d}t$, would be equal to the number of feeders, F, multiplied by the number of unfed ants met by one of these feeders during $\mathrm{d}t$:

$$\mathrm{d}N = F \times [\text{Number of unfed ants met by a feeder in time } \mathrm{d}t] \times \mathrm{d}t. \tag{4.2}$$

The term in brackets in this equation can be broken down further into a rate, R, multiplied by the number of unfed ants, $K - N$:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = F \times R \times (K - N). \tag{4.3}$$

In a physical system consisting of particles which are well mixed, R would be the rate of encounter between any two particles, as in the kinetic theory of gases [128]. In the current context R is taken to represent the rate that a feeder meets unfed ants. If F were proportional to N the equation would result in logistic growth:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = NR(K - N). \tag{4.4}$$

However, we know that the best fit to the data was the recovery exponential, where A, the rate that unfed ants become fed, is a constant:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = A(K - N). \tag{4.5}$$

When equations 4.5 and 4.3 are compared, it is clear that $A = F \times R$. It follows that if A is a constant then $F \times R$ must also be a constant. This can occur in several ways, for example F could increase proportionally as R decreases and vice versa. However, the simplest is if both F and R are constant. Due to the nature of the data collected during this experiment it is not directly possible to determine exact values for F and R. However there is circumstantial evidence, which I will present in the next section, that suggests F and R are approximately constant.

4.7.1 Constant number of feeders, F, and rate of interaction with unfed ants, R

The simplest way in which A can be a constant is if both F and R are constant. A direct measure for the rate R is difficult to extract from the data available as neither the feeding or the spatial data directly measure the rate of all interactions between ants. However there are two pieces of evidence that indicate that R is roughly constant during the famine relief treatment. Firstly, while only a subset of all interactions, the rate of all feeding interactions is constant as a function of time, demonstrated by the linear fit in figure 4-1. Secondly, if we assume that ants are more likely to interact with individuals close to them a proximity based measure can be used as a probability that individuals will interact. Using the spatial point samples for each ant figures 4-13 and 4-14 show the average number of ants that are within a distance x mm to any one ant for $x = 1$ to 4 mm (the bodylength of a worker is roughly 2mm). This average is approximately constant as a function of time with fluctuations, particularly for the smaller values of x, over the 30 minutes in colonies III, IV and VIII. In colony V the average is constant for approximately the first 15 minutes then drops to a lower value and remains roughly constant for the remainder. The first feeding curve for colony V under famine relief actually finishes at $t = 869$ seconds (which is just under 15 minutes), see figure 4-8. This means that for the duration of the first feedings, i.e. $T3 < t < 869$ seconds, the proximity based measure for R is roughly constant. In addition, for colony V the cumulated number of feeding events in figure 4-1 was better fitted by a logarithmic fit, however, between $T3 < t < 869$ seconds the data are best fit by a linear fit with slope 0.308 ($p < 0.005$, R^2=0.991) which also indicates a constant R during this period. Colony VIII under the control treatment shows a large decrease in the average number of ants within 2 to 4

mm of each ant between $t = 10$ and $t = 15$ minutes. There are no obvious clues as to why this occurs, figure 3-7 d.) shows that the number of ants inside the nest remains constant during the 30 minutes. There may have been a cluster of ants at the start of the treatment that spread away from each other later on contributing to the decrease.

If R, the rate of interaction between any two individuals, is roughly constant it implies that F, the number of feeders, is also constant. The exact number of feeders to unfed ants at dt is also difficult to extract directly from the data. However, the number of external ants, i.e. potential foragers and therefore feeders, inside the nest as a function of time is approximately constant, see figure 4-15, with the exception of colony V. The measures I have used here as proxies for F and R include a lot of noise, but it is important to remember that a colony of ants is a biological system and not a physical one so there will tend to be a higher level of noise. In this case N is relatively small and quantized; when one ant does something different it will make a disproportionately large contribution to the noise.

4.7.2 Mixed system

The model that best fits the first feeding data has the assumption that the system is well mixed meaning that all agents move around the arena randomly with the same speed. Each agent is therefore equally likely to interact with every other agent in the system, i.e. every worker inside the nest has an equal probability of interaction with and therefore feeding from every other ant inside the nest. Given what is known about the space use of this species inside the nest, this is a surprising feature to find the colonies expressing if it is found to be true. As described in Chapter 1 *T. albipennis* has a strong spatial structure inside the nest. The structure consists of spatial fidelity zones centered on the brood. Internal workers are therefore unlikely to be well mixed and would encounter some individuals less often than others. For example, a worker who spends the majority of her time very close to the brood is more likely to encounter a worker who also spends her time on the brood than a worker who spends her time in the peripheral regions of the nest. Chapter 5 explores the space use by workers under both treatments and how the well-mixed assumption is met in the real colonies facilitating the famine relief process.

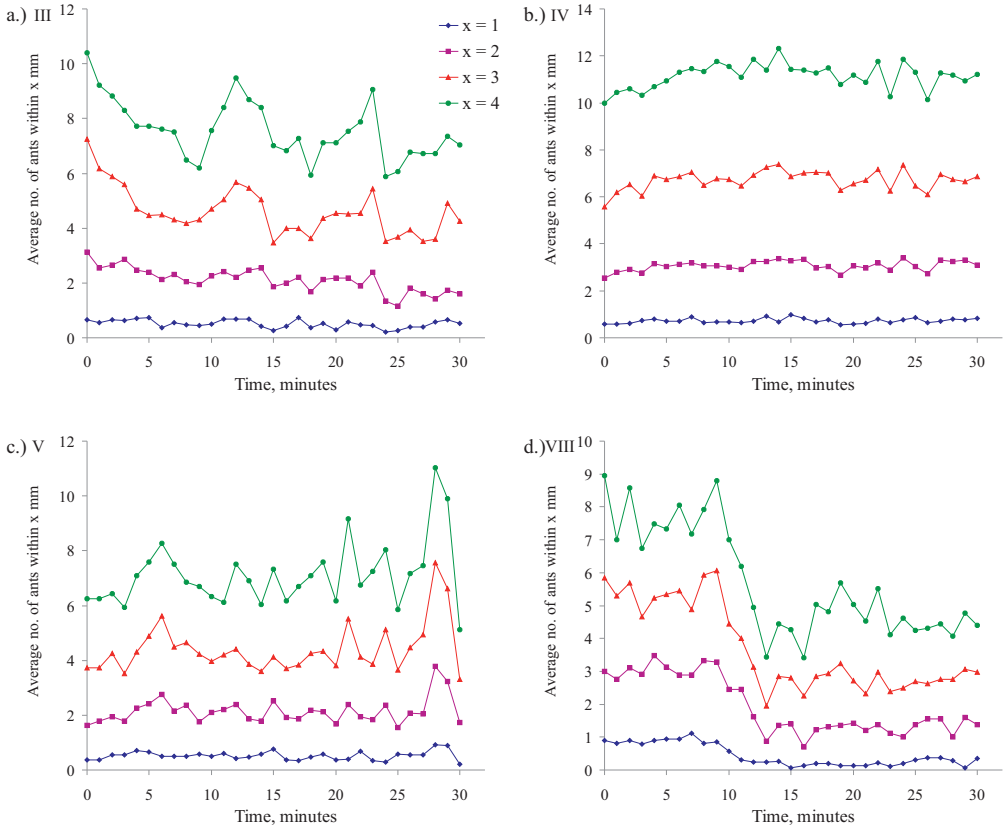

Figure 4-13: *Proximity based measure: Average number of ants within x mm of each ant for $x = 1$ to 4 for the control treatment. Lines between points to guide the eye. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

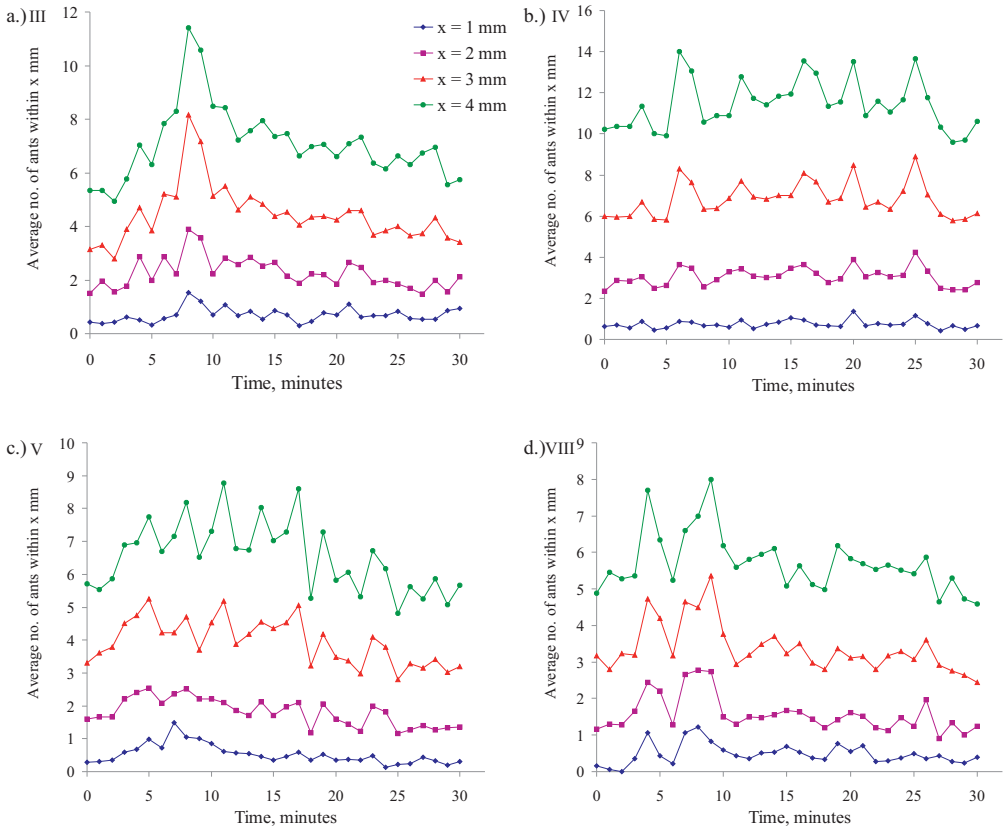

Figure 4-14: *Proximity based measure: Average number of ants within x mm of each ant for $x = 1$ to 4 for the famine relief treatment. Lines between points to guide the eye. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

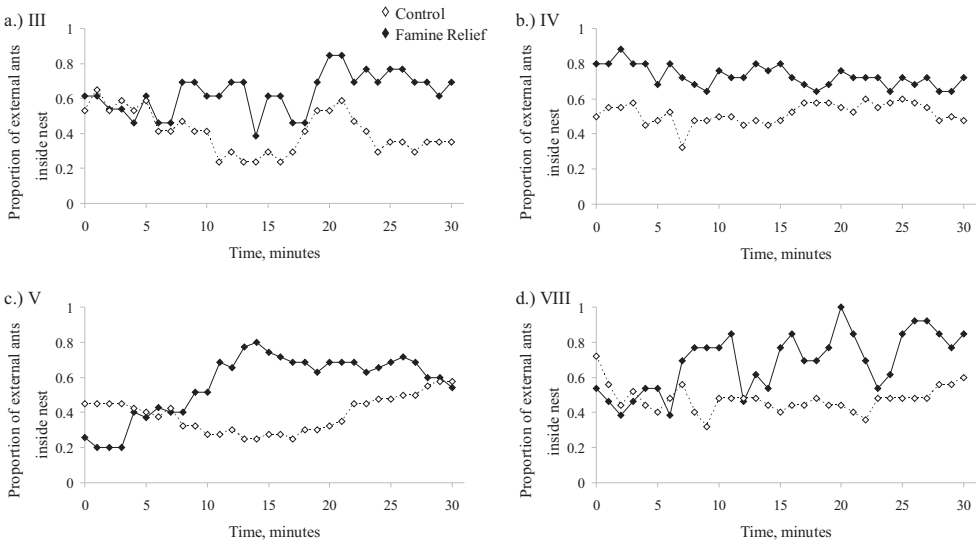

Figure 4-15: *Proportion of external ants that are inside the nest as a function of time. Lines between points to guide the eye, a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

4.8 First feeding networks

We have seen that all four colonies efficiently distribute food to around 95% of the colony during the famine relief treatment. We know that there are some differences between the colonies in how they achieved this, however, so far we do not know the pathways the ants used during this distribution process. Section 4.4 describes how the time an individual first received food is determined. From this first feeding data, if we take the events which represent an individual that is first fed via receiving food during trophallaxis, it is possible to build networks which represent the first time each of these individuals was fed. To ensure that each node in the network has a maximum in-degree of one, ants which are first fed by being a donor are omitted from the networks unless the recipient in the interaction was also unfed. These first feeding pathways are an aspect of the distribution process that cannot be deduced from methods which use labeled food such as in [73, 78].

Figures 4-16, 4-17, 4-18 and 4-19 show the networks of first feedings for each colony under both treatments. There are fewer nodes in the control networks which is not surprising given that many fewer of the ants are fed compared with under the famine relief treatment, see figure 4-8. The networks reveal that under the famine relief treatment while several internal ants, green nodes, are donors

most of the donations are by external ants, i.e. the foragers, orange nodes. In comparison the number of donations from internal and external ants is more equal under the control treatment. Figure 4-20 shows that for ants which donate the average number of recipients that an external ant feeds is much higher than that for an internal ant under the famine relief treatment whereas they are more similar under control (but note the small sample size). However, it is interesting to note that between 10 and 35% of the recipients under famine relief are fed by internal ants. Often this is through an internal ant passing on food, see for example ant 3 in colony III; and occasionally through an internal ant donating food which she must have stored before the start of the treatment, for example see ant 11 in colony III (bottom left in figure 4-16 b.). Under the famine relief treatment there is one ant which is this type of donor in each colony except IV which has nine such donors. These internal ants potentially have stored food, the distribution of which I have called background feeding. The high number of these ants in colony IV is an early indication that there is a high amount of stored food in this colony compared to the other three. These ants are explored in more detail in Chapter 6 including their role in subsequent feeding as well as first feeding.

It is apparent from the first feeding networks that there is a large variation in the number of recipients per donor, particularly clear in colony IV. The out-degree is the number of ants an individual directly feeds. Under control the out-degree distributions for first feedings have a much shorter tail, whereas under the famine relief treatment there are longer tails which indicate a high level of heterogeneity in the out-degree, see figure 4-21. Colonies III and IV for example both have an individual with an out-degree greater than ten (ant 42 in III and ant 83 in IV). (Note that the in-degree for each individual for first feedings is always either 0 or 1.)

One of the aspects of food distribution among workers in ant colonies that has often been inferred from food distribution studies but not directly observed is 'chains of transmission' among individuals [73]. These are created when a recipient passes on food that she has received, in turn becoming a donor herself, e.g. ant 3 in colony III. Because the workers have been individually marked and tracked such behaviour can be shown from this study. In the first feeding networks most of these chains have a pathlength of 1 (no passing on) or 2 (one recipient passes on), and in one instance 3, in colony VIII under famine relief (see ant 39 in figure 4-19). These short path-lengths indicate that the first wave

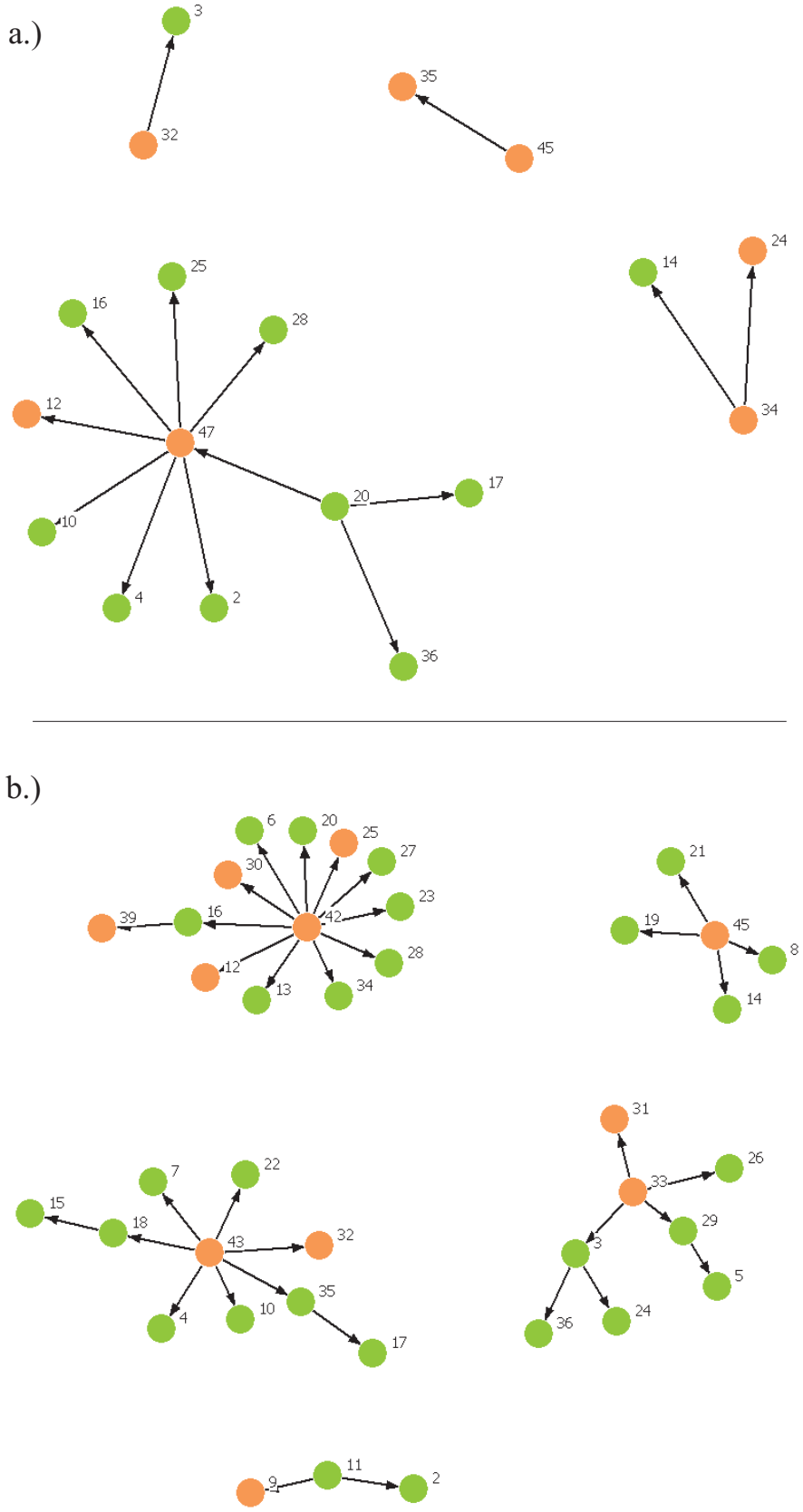

Figure 4-16: *Network of first feedings in colony III. a.) Control treatment, b.) Famine relief treatment. Green nodes represent ants that are internal during that treatment, orange nodes represent ants which are external during that treatment. Nodes are laid out using "Spring embedding" [116] and all interactions shown are causal. Direction of food flow was determined from behavioural observations outlined in section 2.3.2.*

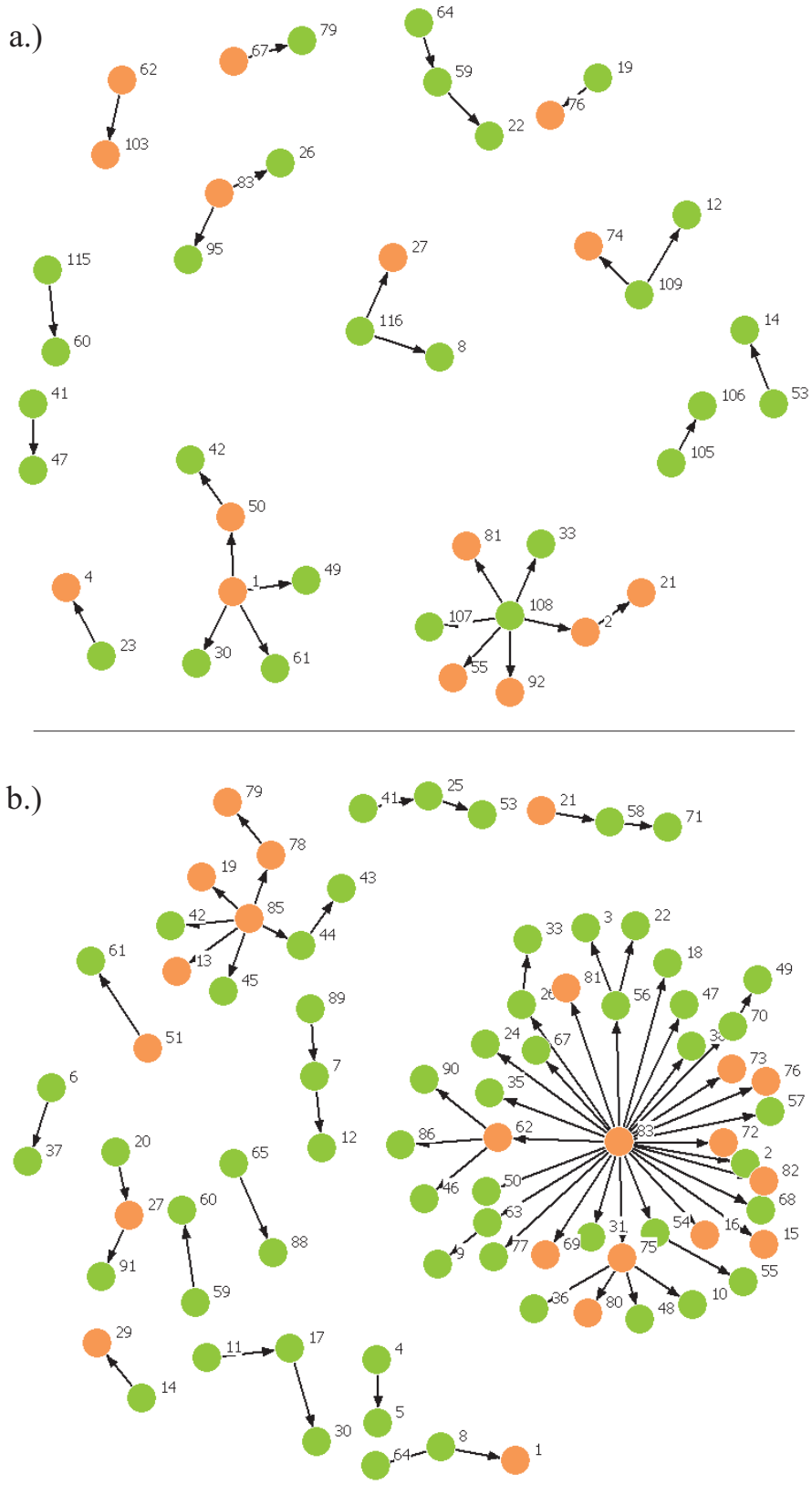

Figure 4-17: *Network of first feedings in colony IV. a.) Control treatment, b.) Famine relief treatment. Green nodes represent ants that are internal during that treatment, orange nodes represent ants which are external during that treatment. Nodes are laid out using "Spring embedding" [116] and all interactions shown are causal. Direction of food flow was determined from behavioural observations outlined in section 2.3.2.*

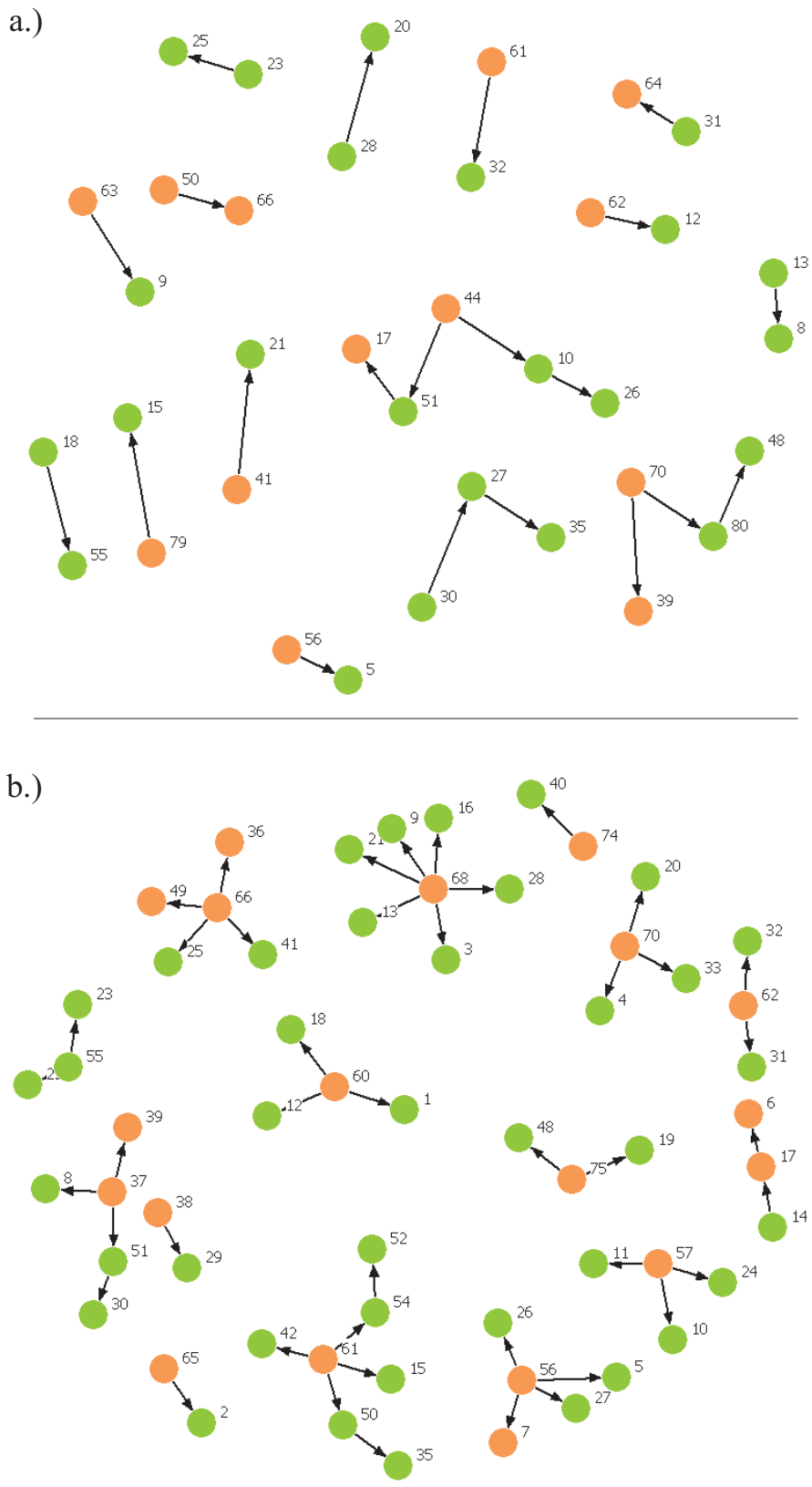

Figure 4-18: *Network of first feedings in colony V. a.) Control treatment, b.) Famine relief treatment. Green nodes represent ants that are internal during that treatment, orange nodes represent ants which are external during that treatment. Nodes are laid out using "Spring embedding" [116] and all interactions shown are causal. Direction of food flow was determined from behavioural observations outlined in section 2.3.2.*

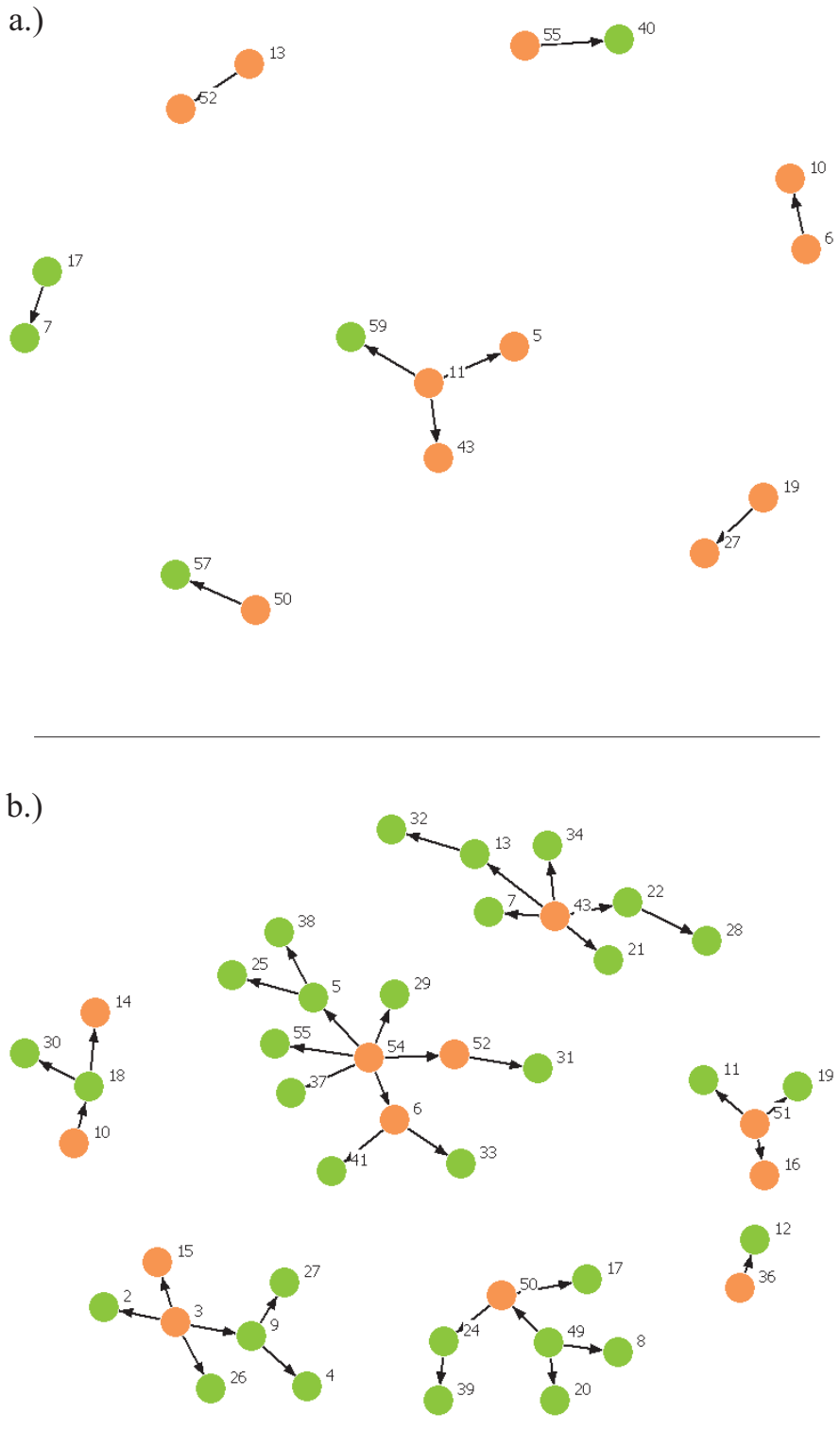

Figure 4-19: *Network of first feedings in colony VIII. a.) Control treatment, b.) Famine relief treatment. Green nodes represent ants that are internal during that treatment, orange nodes represent ants which are external during that treatment. Nodes are laid out using "Spring embedding" [116] and all interactions shown are causal. Direction of food flow was determined from behavioural observations outlined in section 2.3.2.*

of famine relief, the first feedings, are mostly received directly from a forager, a background donor or from an ant who themselves has directly received from a forager or background donor. While not very frequent within the first feeding events longer chains of transmission do occur when we look within all feeding events, this will also be looked at in Chapter 6.

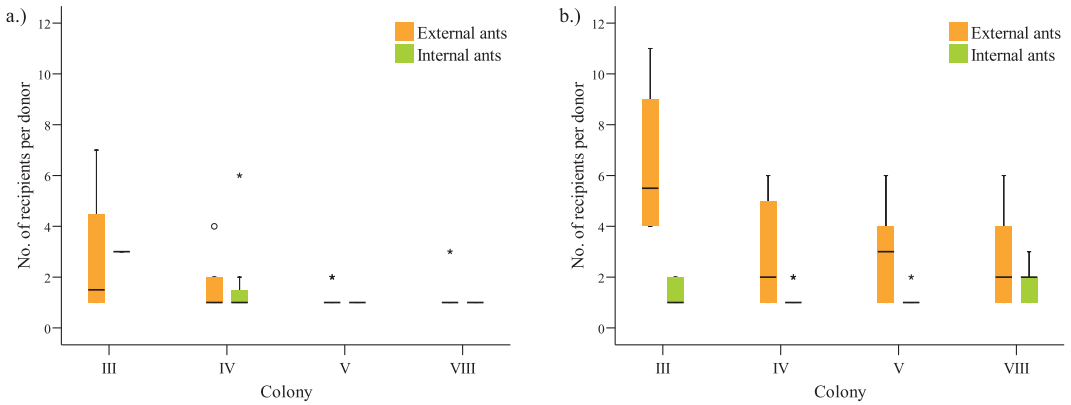

Figure 4-20: *Number of recipients per donor during: a.) Control treatment, b.) Famine relief treatment (One extreme value of 27 for the external ants in colony IV is not shown).*

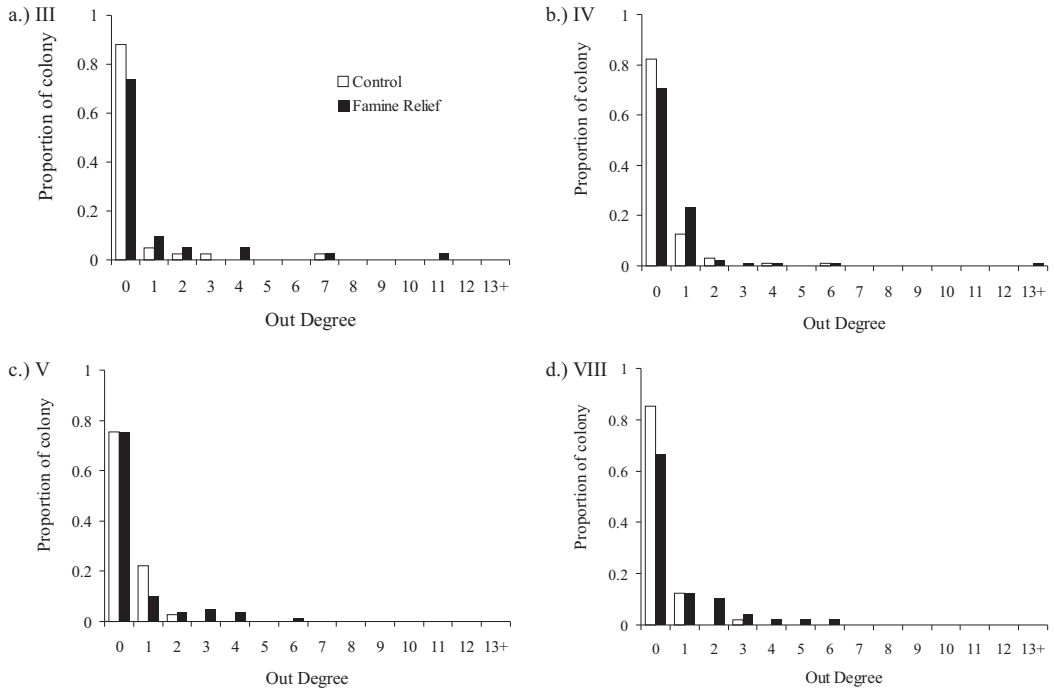

Figure 4-21: *Degree distributions from the first feeding networks. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

4.9 Feeding multiple recipients simultaneously

As seen in the first feeding networks, some of the foragers feed several recipients, for example in colony IV one forager feeds 27 other ants directly (but not all at the same time!). It is not apparent from these networks whether these recipients are fed at the same time or in a series of pairwise events. As explained in Chapter 3, under the famine relief treatment often the feeding events occur with multiple recipients receiving from one donor simultaneously in rosettes. Feeding multiple recipients at once increases the efficiency of the transmission of food in terms of getting food to lots of workers quickly. In Chapter 6 I will explore whether there is a reduced benefit to feeding multiple recipients simultaneously in terms of the amount of food each worker receives.

Feeding recipients together in a rosette also brings the individuals into close proximity of one another. This could increase the ability of an external parasite or pathogen to transmit between individuals which may be disadvantageous. However, as discussed in Chapter 1, exposure to pathogens can also increase future infection resistance, see for example [57]. Figures 4-22 and 4-23 show the average rosette size and the maximum rosette size as a function of time for each colony under the famine relief treatment (there are many fewer rosettes under control, see figure 3-4). There appears to be a trend for the rosette size to decrease over the thirty minutes. Correlation tests show that in colonies V and VIII there is a significant negative correlation between time and rosette size, see table 4.9. The average rosette size is calculated for each feeding event by summing the number of recipients receiving from the donor in that event each second for the duration and dividing by the total duration of the feeding event in seconds. The maximum rosette size for an event is the maximum number of ants simultaneously feeding from the donor during one second. While the result is not significant for all four colonies, there does appear to be a trend for the larger rosettes to occur earlier on during the famine relief treatment. From observing the videos it is apparent that during this phase large rosettes form around returning foragers, then later on in the second 15 minutes the foragers are able to move further into the nest and approach workers. Forming rosettes is a mechanism for distributing food rapidly to multiple individuals during famine relief. The decrease in the number of rosettes seen in the second half of the famine relief treatment in figures 4-22 and 4-23 coincides with the flattening out of the exponential phase of the first feeding curves, see fig. 4-8, i.e. when most ants have received food.

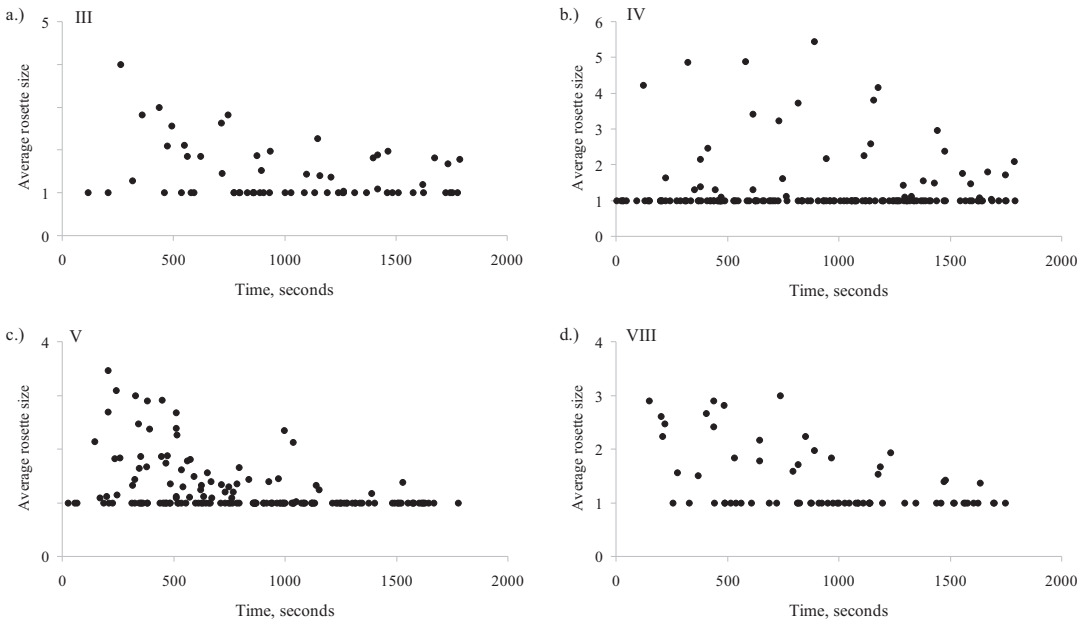

Figure 4-22: *Average rosette size during the famine relief treatment as a function of time, a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

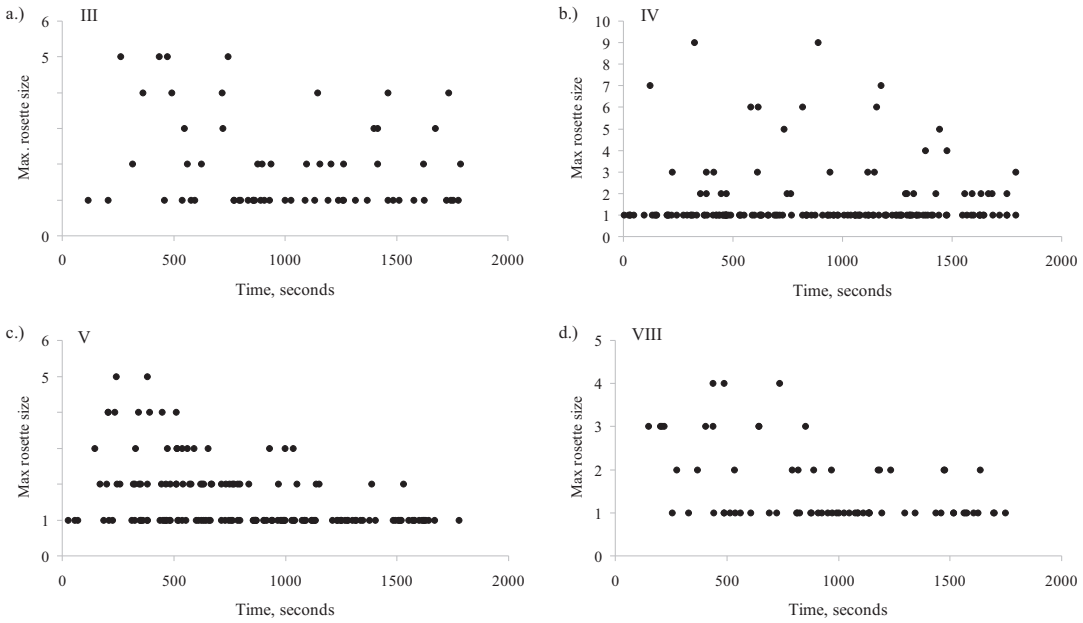

Figure 4-23: *Maximum rosette size during the famine relief treatment as a function of time, a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

	Colony	**Spearman's rho correlation coefficient**	**Sig.**
Av. rosette size	III	0.185	0.303
	IV	0.090	0.252
	V	**-0.433**	**<0.005**
	VIII	**-0.436**	**<0.005**
Max. rosette size	III	0.261	0.142
	IV	−0.061	0.436
	V	**-0.395**	**<0.005**
	VIII	**-0.422**	**<0.005**

Table 4.9: *Spearman's rho correlation coefficients for the rosette size vs. time. All data-sets were non-normal. Entries in bold indicate significant correlations.*

4.10 Subsequent feeding events

At the start of this chapter I explored the overall feeding activity as a function of time. I then went on to describe in more detail the first feeding events. However, between 63 and 76% of the fed ants in each colony receive food more than once during the famine relief treatment; these additional feeding events are referred to as 'subsequent feeding'. Figure 4-24 shows the distributions for the number of times an individual received food in each treatment. The figure shows that during the famine relief treatment many individuals in each colony receive more than three times and in three of the colonies several individuals receive more than ten times. In comparison, under the control treatment of the individuals that receive food most only receive once with a small number receiving more than once. In Chapter 6 I will show that the average amount of food transferred to a recipient during a first feeding event under the famine relief treatment is roughly equal to that transferred during a subsequent feeding event. These additional subsequent feedings may be required to 'top-up' individuals that were not satiated during their first feeding. Distributing the food in multiple receptions of small amounts may be an efficient way to get some food to all individuals quickly. I explore this and other possible concepts in Chapter 6.

From figure 4-8 we know that the first feeding events occur throughout the 30 minutes during the famine relief treatment in colonies III, IV and VIII, and during the first 15 minutes in colony V. Given that so many individuals receive additional food it is interesting to know how much of this subsequent feeding occurs while much of the first feeding is still in progress. Figure 4-25 illustrates the proportion

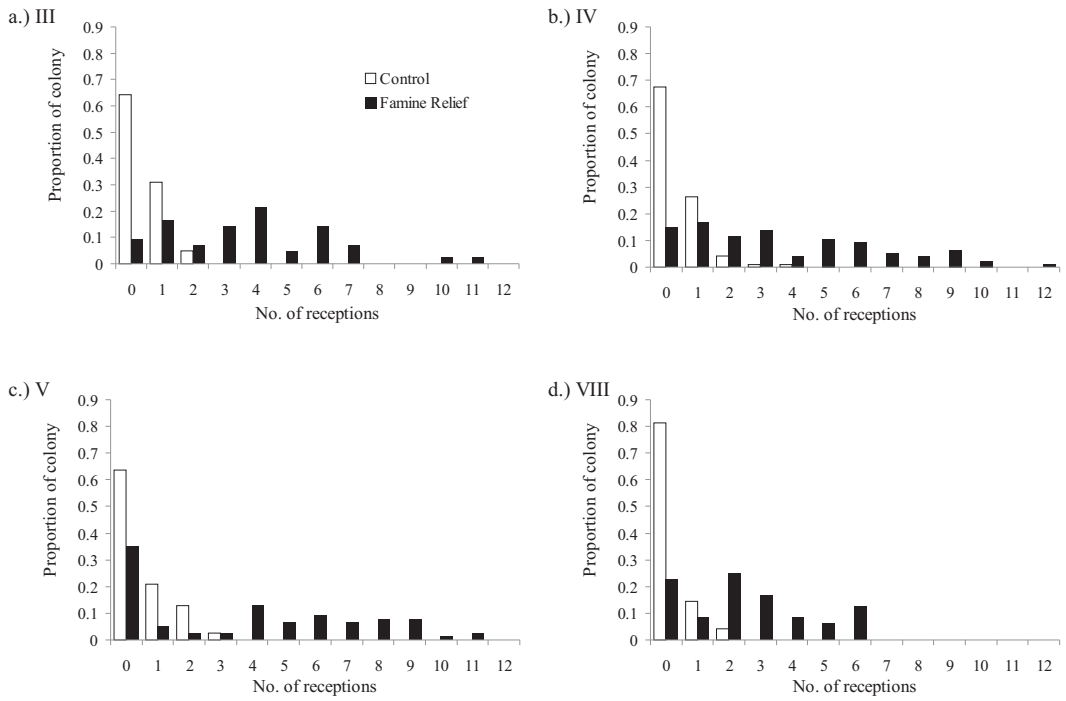

Figure 4-24: *Distributions for the number of times individuals received food during each treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

of ants in each colony that are receiving subsequent feeding averaged over the 30 minutes for each treatment. It shows that this average is similar across all four colonies under the famine relief treatment and is very low under control. Figures 4-26 and 4-27 show the food receiving activity as a function of time as in figures 4-3 and 4-4 but split into first and subsequent feeding. It appears that during the famine relief treatment in colonies III and V there is a switch from mostly first feeding to subsequent feeding at approximately 900 seconds in III and 400 seconds in V. In contrast, in colonies IV and VIII apart from at the very start where all feedings must be first feedings, there is not such a clear switch. In these two colonies the proportion of ants receiving in first feeding events does not show the same decrease as in III and V. It also appears as though the level of subsequent food receiving activity in colonies V and VIII mirrors the first feeding with a delay and some amplification whereas in colonies III and IV the subsequent food receiving activity does not follow a similar pattern to the first food receiving activity.

A linear regression was performed on the proportion of ants receiving subsequent feeding as a function of time to verify the existence of a switch between first

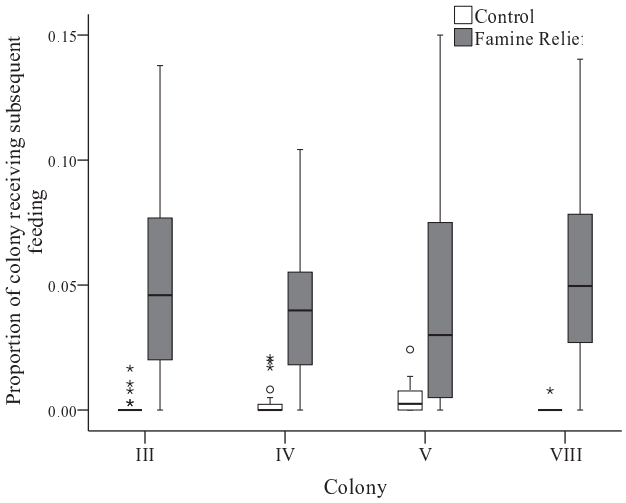

Figure 4-25: *Proportion of the colony receiving in a subsequent feeding event at any one time during the 30 minutes of each treatment (i.e. not first feeding events).*

Treatment	Colony	Gradient	Sig.	R^2	Constant
	III	**0.003**	**0.000**	**0.435**	**0.007**
Famine	IV	0.001	0.084	0.100	0.023
Relief	V	**-0.002**	**0.009**	**0.212**	**0.083**
	VIII	0.001	0.319	0.034	0.040

Table 4.10: *Results from the regression analysis with proportion of colony receiving subsequent feeding as the dependent and time as the independent variables. Bold entries indicate significant results.*

feeding and subsequent feeding. The results of this analysis are shown in table 4.10. They show that in colonies IV and VIII there is no significant trend for this proportion to increase or decrease with time verifying that there is no switch between the two types of feeding. In colony III there is a significant positive gradient in the proportion of ants receiving subsequent feeding which verifies the switch from first feeding to subsequent feeding. In colony V, however, the regression analysis shows a significant negative gradient in the proportion receiving subsequent feeding. Looking back to figure 4-27 the food receiving activity in V decreases with time altogether so it is not surprising that the subsequent feeding also shows this trend.

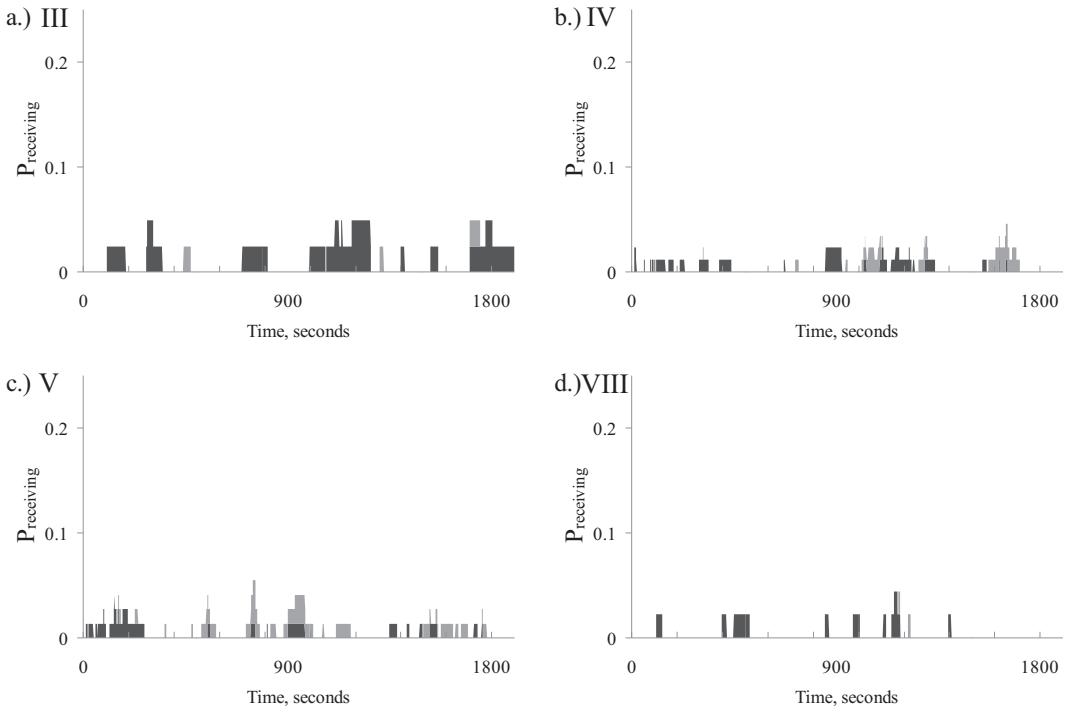

Figure 4-26: *Proportion of ants receiving in first feeding, black, and subsequent feeding, grey, under the control treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

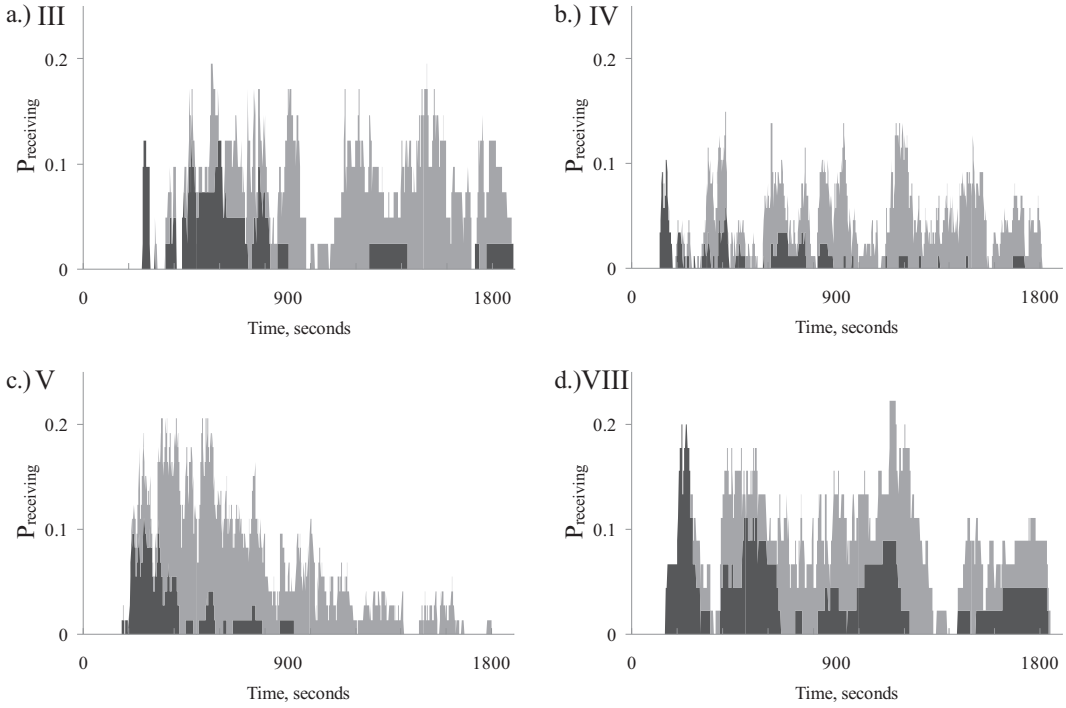

Figure 4-27: *Proportion of ants receiving in first feeding, black, and subsequent feeding, grey, under the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

4.11 Summary

In this chapter I have explored the distribution of food from the temporal aspect focusing on objective A: comparing the rates of feeding between the two treatments and how faster feeding might be achieved during famine relief. The rates of all feedings and first feedings are an order of magnitude higher under famine relief compared with under control, see figures 4-1 and 4-8. During control overall activity levels were expected to be low as typically over 50% of a colony tend to be inactive at any one time [129]. Meanwhile, when food is introduced after a period of starvation activity levels are expected to be high, particularly feeding activity, seen by the rapid transmission of food in other studies, e.g. [73, 78] (although these studies did not compare with rates of feeding under normal conditions). This rapid transmission of food during famine relief has implications for how a pathogen carried in the food might spread and later we will look at how the colonies might be structuring their transmission pathways to reduce this. The recovery exponential gave the best fit for the first feeding curves (figure 4-8), a result that has also been found in *Formica fusca* [78]. This suggests that perhaps the organisation of food transmission inside the nest is similar across ant species. One of the assumptions of a model which is consistent with a recovery exponential fit is that the system is well mixed. This is an interesting result given that this species demonstrates a strong spatial structure inside the nest [107, 95, 108]. We will look at whether this spatial structure is maintained during famine relief in Chapter 5; abandoning the structure may be one way in which the colonies achieve higher rates of transmission during famine relief.

Another way to achieve higher rates of feeding during famine relief would be to increase the speed with which workers move and therefore interact as shown in Chapter 3 figure 3-6. In this chapter I have shown evidence that the colonies are also changing their behaviour in other ways to facilitate the rapid transmission of food for example by feeding more ants simultaneously in rosettes towards the start of the famine relief treatment, figures 4-22 and 4-23. Feeding multiple recipients simultaneously is an efficient way to get food to many workers at once and is seen in other ant species after starvation [79]. However, it presents yet another condition which potentially favours the spread of a pathogen by bringing many individuals into close proximity of one another [41, 2].

Analysis in this chapter revealed that more of the donations that relieved the famine (first feedings) were carried out by foragers than non-foragers, figure 4-20.

This suggests that the initial 'wave' of famine relief to most ants comes directly from foragers. This may allow foragers to gather as much information as possible with regards to the hunger level of the colony by interacting with many nestmates and for the nestmates to receive information about the presence of the newly found food.

Differences in how the colonies achieve the efficient distribution of food during famine relief have been uncovered, for example: the level of food receiving activity as a function of time, figure 4-4; differences in the proportion of workers outside the nest, figure 4-5; and the distributions of number of receptions per ant, figure 4-24. We know that there is variation between the colonies in their demographic and geometric properties, see section 3.1. The differences in behaviour may arise from this pre-existing variation and we can begin to piece together the different strategy each colony is using. We know that colony IV is the largest both in terms of the number of workers but also the size of the brood pile. The first feeding in this colony is mostly undertaken by one forager; in contrast in colony V, the second largest colony, the first feeding network is much more fragmented. In this colony many more of the workers leave the nest in both treatments compared to the other three colonies, see figure 4-5, resulting in a higher number of foragers. This contributes to the high level of food receiving activity and faster rate in the first feeding curve during the first 15 minutes in this colony compared to the other three, see figure 4-8. The relatively small brood in colony V may permit or even drive a large proportion of the workers to leave the nest and forage for food themselves. The two smaller colonies appear to be fairly similar so far; however there is evidence that colony VIII uses fewer receptions but of longer duration; this will be explored further in Chapter 6.

Chapter 5

Food transmission from a spatial perspective

This chapter primarily addresses objective B: to explore the space use of the workers during the two treatments and determine whether the spatial structure is maintained or abandoned during famine relief. The spatial organisation of colony members inside the nest potentially plays a key role during food distribution. It has been shown that the workers of *T.albipennis* are organised into zones or stations in which they spend the majority of their time within the nest [95, 107]. During food distribution foragers might adapt their space use to reach workers deeper in the nest or the workers might transfer food on to their nearest neighbours creating chains of transmission based on spatial proximity. Conversely the distribution process itself may influence the space use of the workers who might facilitate more efficient food sharing by abandoning their spatial fidelity zones. In addition to the spatial organisation of the workers the geometric features of each colony, particularly the size and location of the brood pile, may also influence the organisation of food distribution.

In Chapter 4 it was shown that the four colonies resolved the famine efficiently, see figure 4-8, and a model that was consistant with the data assumes that agents are well mixed within the system. This would imply that the workers abandon their spatial structure (which is not well mixed) during famine relief to facilitate faster feeding to unfed workers. One of the aspects that I will focus on in this chapter is whether the ants adhere to their spatial fidelity zones during the famine relief treatment. The control treatment was designed to represent typical conditions

inside the nest while in the laboratory. Given that the conditions experienced prior to and during the famine relief treatment are different from this we may expect the colonies at some point to return to the spatial organisation seen under the control treatment. However, this might not necessarily occur within the thirty minutes analysed from the famine relief treatment.

In previous chapters we have already uncovered several common features of space use across all four colonies including: a higher proportion of workers outside the nest during the control treatment; an increase in 'straight-line speeds' of internal workers during the famine relief treatment; and an apparent temporary abandonment of brood pile towards the start of the famine relief treatment. To determine the change in space use this chapter includes comparisons between the two treatments of: the intensity of space use within the nest; centre of mass of the ants as a function of time; individual and total effective areas of space use (determined using convex polygons); and overlap of individual areas. This chapter also includes a categorisation of the ants in each colony by their space use for later analysis in Chapter 6 and the final spatial distribution of food within the nest for comparison with the result of a recent study in the ant *Formica fusca*, see [78]. We will see that the ants do not use the whole area inside the nest, instead they mostly use a smaller effective area. The intensity of space use within this smaller area and the centre of mass of the workers differs between the two treatments. The ants expand their individual area of space use, move away from the brood and into the arena close to the nest entrance at the start of the famine relief treatment facilitating the higher rate of feeding.

As described in Chapter 2 spatial information about each colony member under both treatments was obtained by manually tracking each individual in turn and recording its location inside the nest every minute for the first 30 minutes of each treatment. This yields 31 data points, referred to as Spatial Point Samples, SPS, for each ant per treatment. When an ant is outside the nest for a SPS her location is represented as $x = 20$mm and $y = 40$mm, an arbitrary point outside the nest (Note: originally recorded as $x = 100$ and $y = 100$ in AntTracker). The information from the positions of the brood items is also used in this chapter.

5.1 Colony level

From observations of wild colonies and studies on laboratory kept colonies we know that workers of *T.albipennis* are not uniformly distributed over the entire

area inside the nest cavity, [95, 107], and see figures 1-1 and 3-1. The density of workers is higher towards the centre of the nest, generally where the brood pile and queen are located. It is also known that in this species workers will allow approximately 5mm^2 per ant when permitted to build the nest wall [92]. The artificial nests used in this experiment (39 × 32mm) allow a much greater area per ant, ranging from 13 to 30 mm^2 per ant depending on colony size, and no building materials were provided. Given that a much larger area per ant is available, before we begin to investigate the space use at the individual level it is useful to know whether the colonies use the whole area inside the nest or whether they restrict their space use to a smaller effective area. The SPS for all ants in each colony cumulated over the 30 minutes of each treatment can be used to calculate the intensity of space use over the area inside the nest. Figure 5-1 shows contour plots for the intensity of space use by the ants inside the nest. These plots were made by splitting the area filmed (i.e. 45 × 36mm, the nest cavity plus border) into a 20 by 20 grid and counting how many SPS points were in each square of the grid. I then normalised the counts by the total possible number of SPS, i.e. 31 × number of tracked ants in the treatment, taken from table 2.2, and used MatLab to create contour plots.

Figure 5-1 shows that the colony level space use is similar under the two treatments for all four colonies but that the area used under famine relief expands slightly compared to under the control treatment. Under the control treatment there appear to be two hot-spots where ants are concentrated: one centered on the brood pile and one close to the nest entrance. When the contours are compared between treatments for each colony we can see that these hot-spots become less concentrated under the famine relief treatment and the area between the nest entrance and the brood pile becomes more occupied. This pattern can be quantified by looking at the centre of mass of the ants within the nest to see if there is a shift away from the brood and towards the nest entrance under the famine relief treatment.

5.1.1 Centre of mass of ants as a function of time

We now have several pieces of evidence that the ants alter their spatial behaviour between the two treatments. In section 3.3.1 I described how there was a general trend in the famine relief treatment for workers to leave the brood during the

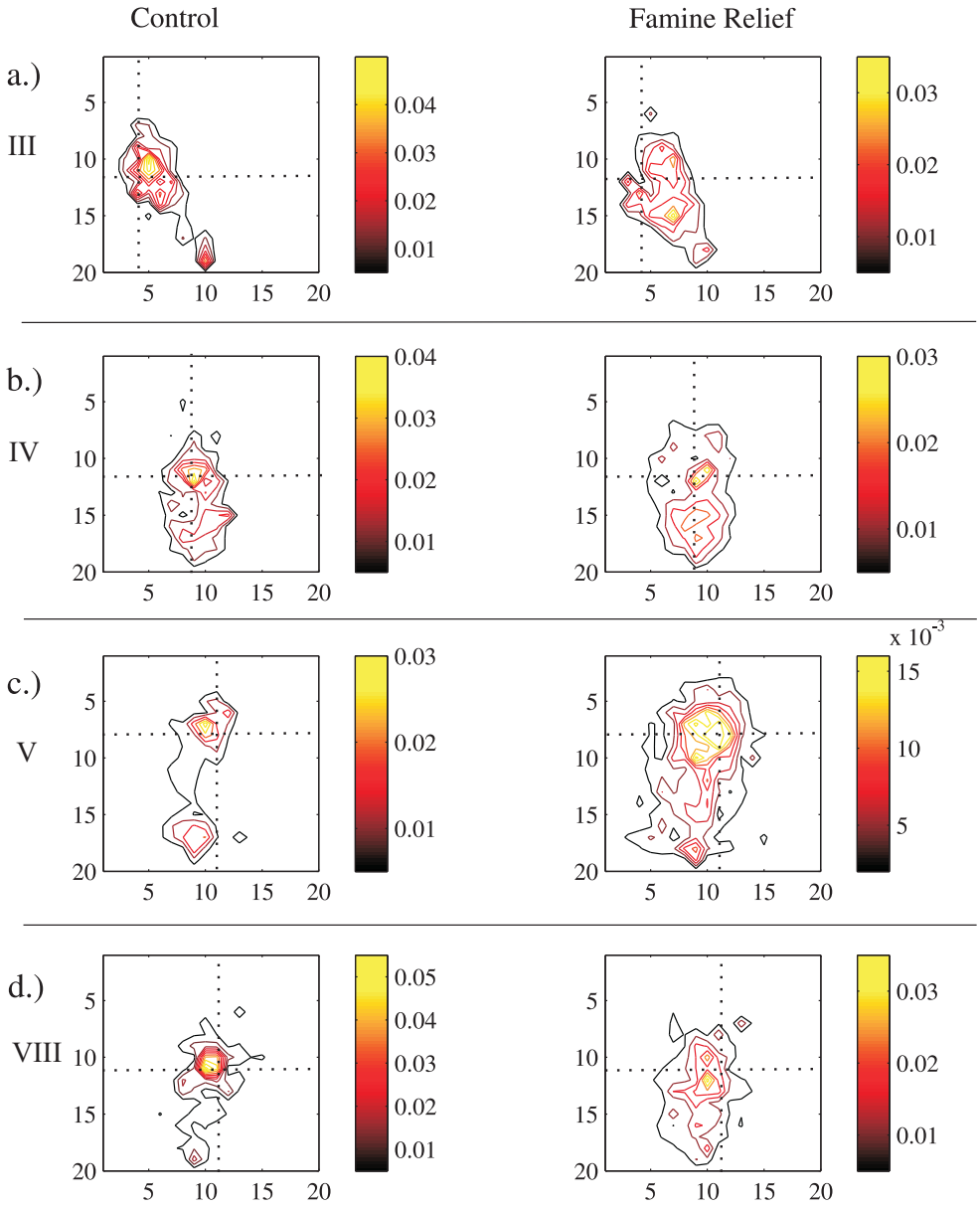

Figure 5-1: *Intensity plots of space use inside the nest with the control treatment on the left and the famine relief treatment on the right. Intersection of the dashed lines represents the centre of the brood pile. The nest entrance is at approximately (10,20) in all cases. 1 unit in the x direction is equal to 2.25 mm while 1 unit in the y direction is equal to 1.8 mm. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

'frenzy', see figure 3-7. This abandonment of the brood appears to be only temporary shown by the subsequent increase in brood coverage. However, there may be a pattern over the whole 30 minutes that shows a change in space use between the two treatments that can be detected by examining the centre of mass of the ants.

I have classified all ants as either internal or external ants for each treatment and calculated the centre of mass for these groups as well as for all ants together. Internal ants are defined as those who had all 31 SPS inside the nest during that treatment, whereas external ants had at least 1 SPS recorded as outside the nest. As these two groups fulfill different roles we may expect there to be a difference in space use between them which might not be apparent if they are grouped together.

The centre of mass is calculated by averaging the coordinates of the SPS of the ants inside the nest at that minute. I then calculated the distance between the centre of mass of the ants and the centre of the brood pile. This distance is important because the brood pile is considered the biological centre of the colony [95], and the 'hotspot' of workers appears to shift slightly away from the brood during the famine relief treatment, see figure 5-1. The centre of the brood pile is calculated by averaging the coordinates of the brood items recorded in section 2.3.3.

Figure 5-2 shows the average distance between the centre of mass and the centre of the brood pile for each of the three groups: all, internal and external ants. The figure shows that under both treatments the centre of mass of the external ants is further from the centre of the brood pile than for the internal ants. The figure also shows that in all four colonies the distance between the centre of mass of the internal and external ants decreases under the famine relief treatment. This indicates that the two groups are becoming closer together which would facilitate the flow of food from the external ants to the internal ants. This reduction in distance between the two groups is achieved by the internal workers moving away from the brood in all four colonies. In colonies III and V, where the reduction in distance is largest, the external workers also show a shift towards the brood.

Figures 5-3 and 5-4 show the centre of mass of ants as a function of time to see if there is a temporal pattern to this change. A linear regression was carried out on each data set, see tables 5.1, 5.2 and 5.3. The clearest pattern is that during the

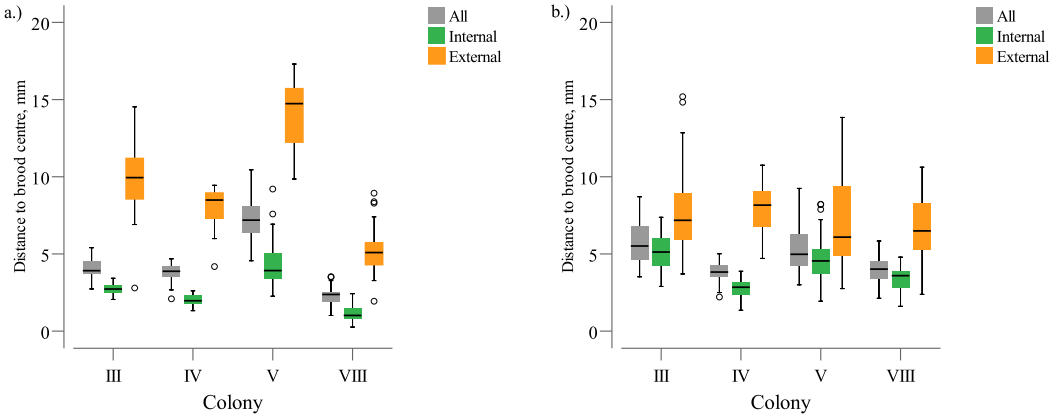

Figure 5-2: *Distance in mm between the centre of mass of ants and the centre of the brood pile, a.) Control treatment, b.) Famine relief treatment.*

famine relief treatment three colonies, III, V and VIII, have significant negative gradients for all groups. This shows the ants are moving closer to the brood pile as a function of time. The distance between the ants and the centre of the brood pile at the start of the famine relief treatment in these colonies is larger than that under the control treatment. The internal workers could be returning to their typical spatial organisation seen under control once the initial wave of famine relief has finished while the foragers enter further into the nest and closer to the brood pile to feed remaining unfed ants. In colony IV under the famine relief treatment the ants actually move slightly further away from the brood pile shown by a significant positive gradient. This may be explained by the fact that all the ants were closer to the centre of the brood at the start of the treatment compared with during control. Thus to return to the typical conditions seen during the control the workers must move slightly away from the centre of the brood.

The results for the control condition are less clear. Colony III shows no significant gradient as we expect. Colonies IV and V both show a positive gradient for the internal (and all) ants (but not external). Perhaps during times of food abundance the workers in these colonies undertake tasks slightly further away from the brood. Colony VIII shows internal ants getting closer to the brood and external ants getting further away. These differences during the control treatment across the four colonies may indicate different management strategies during times of food abundance. However, it is important to note that the R^2 values for these results are generally low for the significant results, ranging from 0.155 to 0.768.

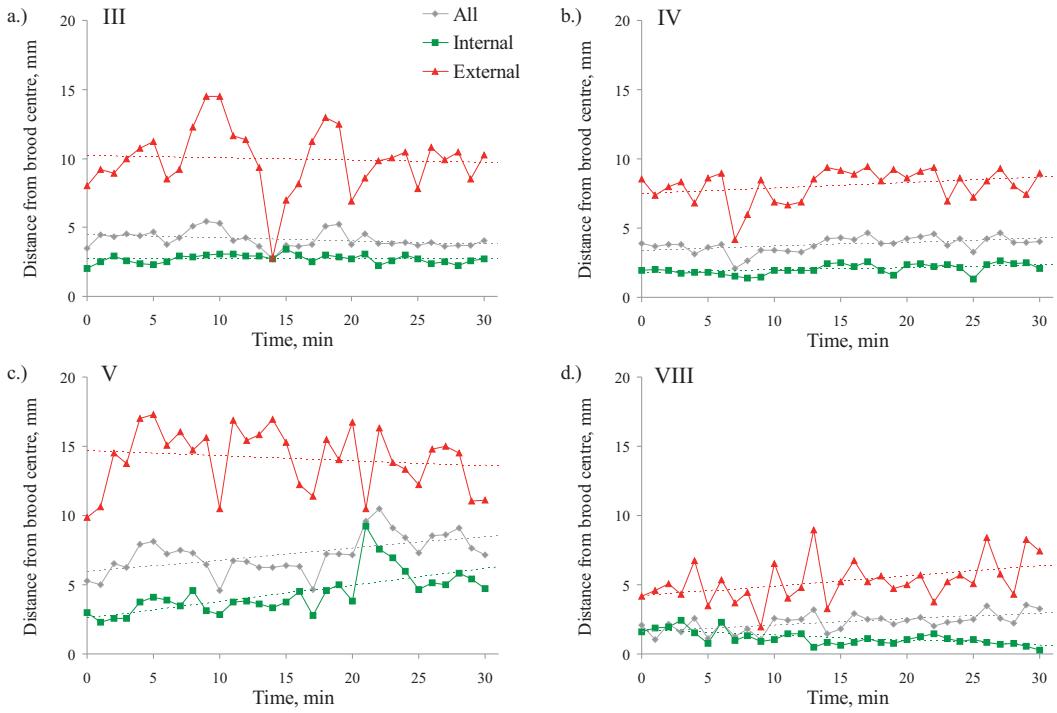

Figure 5-3: *Centre of mass of ants as a function of time under the control treatment. Lines between points to guide the eye, dashed lines represent linear fits, a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

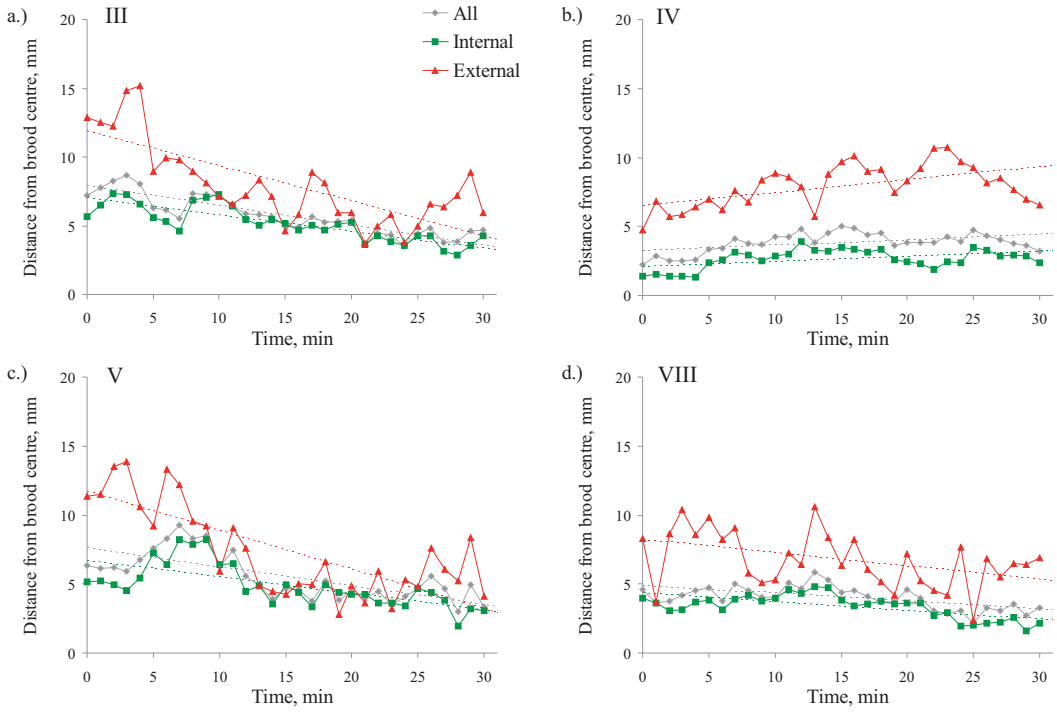

Figure 5-4: *Centre of mass of ants as a function of time under the famine relief treatment, Lines between points to guide the eye, dashed lines represent linear fits, a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

Treatment	Colony	R^2	Sig.	Gradient	95% CI for gradient
Control	III	0.094	0.093	−0.021	0.024
	IV	**0.224**	**0.007**	**0.030**	**0.020**
	V	**0.284**	**0.002**	**0.082**	**0.047**
	VIII	**0.364**	**0.000**	**0.044**	**0.022**
Famine Relief	III	**0.768**	**0.000**	**-0.138**	**0.027**
	IV	**0.236**	**0.006**	**0.039**	**0.025**
	V	**0.503**	**0.000**	**-0.131**	**0.047**
	VIII	**0.338**	**0.001**	**-0.053**	**0.027**

Table 5.1: *Gradients of the linear regression analysis of distance of centre of mass of all ants from centre of mass of brood against time. Bold entries indicate significant results at the* $p < 0.05$ *level.*

Treatment	Colony	R^2	Sig.	Gradient	95% CI for gradient
Control	III	0.000	0.994	−5.3E−05	0.012
	IV	**0.270**	**0.003**	**0.021**	**0.012**
	V	**0.437**	**0.000**	**0.112**	**0.047**
	VIII	**0.424**	**0.000**	**-0.037**	**0.016**
Famine Relief	III	**0.687**	**0.000**	**-0.116**	**0.029**
	IV	**0.187**	**0.015**	**0.033**	**0.025**
	V	**0.447**	**0.000**	**-0.144**	**0.045**
	VIII	**0.408**	**0.000**	**-0.059**	**0.025**

Table 5.2: *Gradients of the linear regression analysis of distance of centre of mass of internal ants from centre of mass of brood against time. Bold entries indicate significant results at the* $p < 0.05$ *level.*

Treatment	Colony	R^2	Sig.	Gradient	95% CI for gradient
Control	III	0.004	0.745	−0.016	0.092
	IV	0.080	0.124	0.037	0.045
	V	0.018	0.471	−0.033	0.090
	VIII	**0.155**	**0.028**	**0.068**	**0.057**
Famine Relief	III	**0.548**	**0.000**	**-0.244**	**0.080**
	IV	**0.278**	**0.002**	**0.090**	**0.053**
	V	**0.553**	**0.000**	**-0.269**	**0.088**
	VIII	**0.180**	**0.017**	**-0.094**	**0.073**

Table 5.3: *Gradients of the linear regression analysis of distance of centre of mass of external ants from centre of mass of brood against time. Bold entries indicate significant results at the* $p < 0.05$ *level.*

So far we have seen that ants abandon the brood at the start of the famine relief treatment in order to receive food from incoming foragers, figure 3-7 in Chapter 3. I have just shown that the ants gradually return to the brood pile throughout the famine relief treatment corresponding with most ants resuming their normal positions once they have been fed. The space use in the remainder of the nest will be investigated in the following section. Because there is only one entrance to the artificial nest and a brood pile the area inside the nest is asymmetric and inhomogeneous. I will use convex polygons formed from the SPS data to investigate how the space inside the nest is used. First I will outline how these convex polygons are formed.

5.2 Individual level

5.2.1 Forming convex polygons from the spatial data

The spatial results presented so far have mostly been one-dimensional, for example distance from the brood centre. However, the SPS give us a much richer source of information about how the ants use the space inside the nest. For each treatment there are at most 31 SPS points inside the nest per ant. To give an impression of the area used by each ant we can draw a convex polygon around the outer most of these points. I wrote an algorithm in Fortran that does this based on Graham's algorithm [130]. From these convex polygons I can calculate an area of space use for each ant, see where the polygons are inside the nest and how much they overlap with the polygons of other ants.

If we look at the SPS for an ant from each colony we see that occasionally there are points that are clearly not representative of their space use for the majority of the time, see figure 5-5. Outliers in this respect may be important in some contexts, such as calculating maximum distance an ant travels in the nest or the maximum area covered inside the nest. However they are not vital for evaluating representative space use and may in this case lead to a misrepresentation of the area that an ant mostly uses. Therefore for each ant I have calculated the centre of mass of the SPS that are inside the nest for that ant and used the points closest to this centre of mass to form the convex polygon. An ant can have a maximum of 31 SPS inside the nest; I have removed the 15 SPS furthest from

the centre of mass (that are inside the nest) and used the remaining points to construct a 'Median Convex Polygon', MCP. This means that for the external ants sometimes the polygons are based on only a few points inside the nest and sometimes there are too few points left to form a polygon, i.e. ants with less than 18 SPS inside the nest will not have a polygon. I have chosen to do this so that an ant which is mostly outside the nest is not represented by a disproportionately large polygon formed from the 4 SPS when the individual was inside the nest. Due to this most of the analysis using the MCPs will be based on the internal ants only.

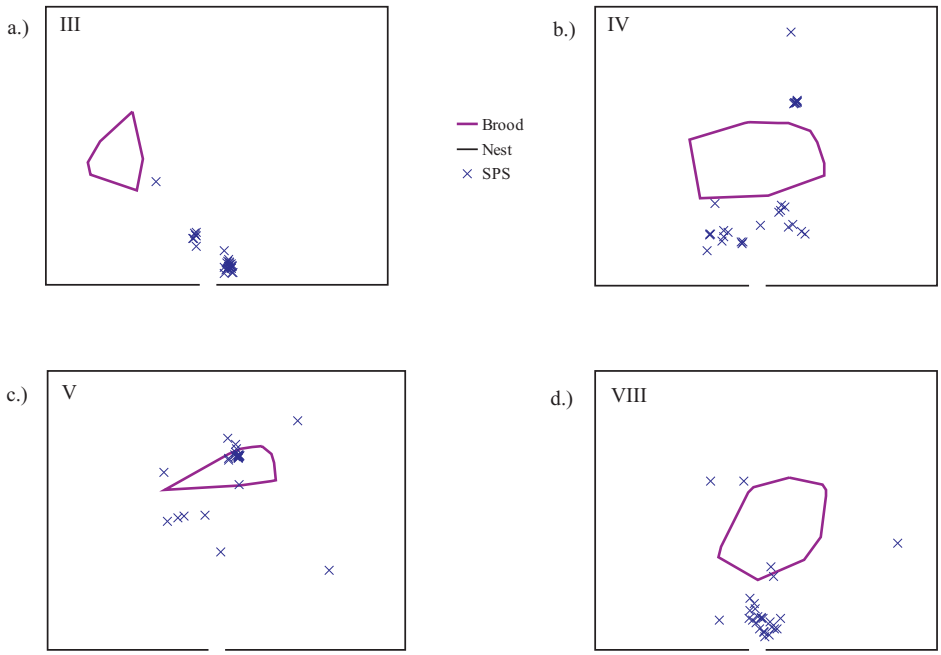

Figure 5-5: *Examples of the Spatial Point Samples, SPS, inside the nest for one ant from each colony highlighting that often there are a few outliers that are not representative of where an ant spends the majority of its time. Purple polygons show the convex polygon formed around the brood items in each colony. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

Figure 5-6 shows the MCPs for all individuals in each colony for the two treatments. There is a striking difference between the polygons of the two treatments. Under the control treatment, shown on the left, the polygons are smaller and less overlapping whereas under the famine relief treatment, shown on the right, the polygons are much larger and overlap with each other much more.

The contours in figure 5-1 give some idea of the effective area used inside the nest. However, we can use the SPS for all ants to form a convex polygon which

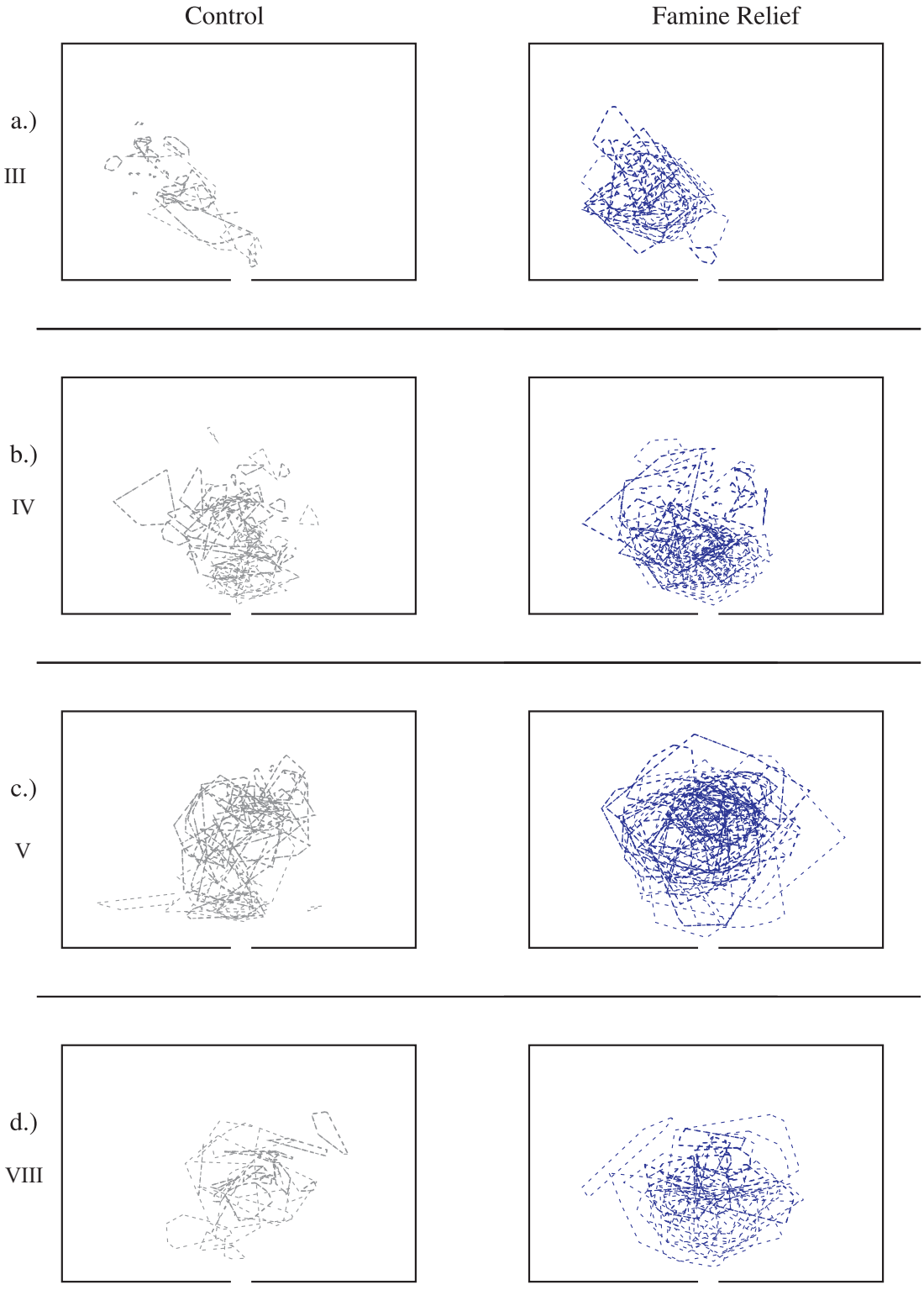

Figure 5-6: *Median Convex Polygons, MCPs, of space use for all ants. Control treatment is shown in the column on the left, famine relief treatment is shown in the column on the right. Black rectangle represents the nest outline, the gap indicates the entrance. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

represents this effective area. Again this was formed using the median number of SPS closest to the centre of mass of all the SPS for internal ants in each treatment. These polygons are shown in figure 5-7 which shows that the areas of the polygons are similar for colonies III and IV, whereas for colonies V and VIII the polygon for famine relief is much bigger than that for control, highlighted in table 5.4. It is interesting to note that colony IV has a much higher density of internal ants within the effective area polygon than the other three colonies under the famine relief treatment. This is likely to restrict the movement of the workers resulting in the lower speeds seen previously in figure 3-6.

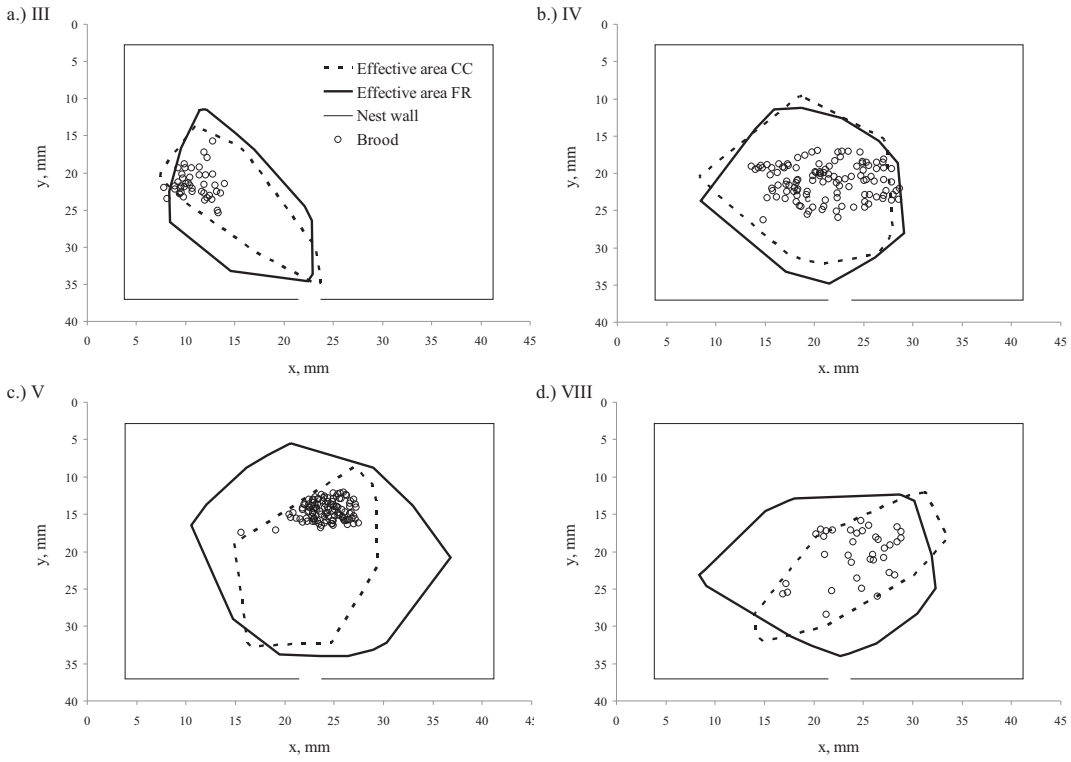

Figure 5-7: *Polygons of effective area used by internal ants with brood items shown for each colony. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

5.2.2 Areas of "Median Convex Polygons", MCPs

The Median Convex Polygons, MCPs, can be used to look at the area, location and overlap of space use by individuals within a colony. Figure 5-8 shows the distributions of the areas of the MCPs in all four colonies. The figure shows that under the control treatment in three of the colonies the areas are mostly between

Treatment	**Colony**	**Effective area, mm²**	**Density within effective area, ants per mm²**
Control	III	148.8	0.168
	IV	289.1	0.169
	V	246.1	0.146
	VIII	192.8	0.104
Famine Relief	III	215.4	0.135
	IV	326.7	0.187
	V	508.5	0.079
	VIII	358.4	0.095

Table 5.4: *Effective area and density of internal ants for all four colonies under the two treatments.*

10 and 30 mm^2, in colony V the spread is a lot wider ranging up to 100 mm^2. In comparison under the famine relief treatment the areas in all four colonies range to above 100 mm^2 and there is a large decrease in the proportion of MCPs that have an area between 0 and 10 mm^2. I constructed a 2 by 6 contingency table for each colony using the two treatments as the columns and areas in bins of $10mm^2$ up to $50mm^2$ as the first five rows with the sixth row for areas greater than $50mm^2$. A chi-squared test for goodness of fit on these contingency tables for each colony shows that there is an association between treatment and area (χ^2: III = 16.76; IV = 16.37; V = 29.07; VIII = 17.37, all significant at the $\alpha < 0.01$ level for 5 degrees of freedom). This demonstrates that the distribution of areas of individual space use under the famine relief treatment is significantly different to that under the control treatment in all four colonies. Meanwhile, the phi-coefficient, $\phi = \sqrt{\chi^2/N}$ [131], shows the strength of this association between treatment and area is strongest in colony V and weakest in colony IV (ϕ: III = 0.504; IV = 0.330; V = 0.524; VIII = 0.488; where values close to 0 correspond to no association and values close to 1 correspond to strong association). This means that the largest difference in the distribution of MCP areas between the control and famine relief treatment is in colony V. The small brood pile far from the nest entrance in this colony may allow the workers to use a much greater area within the nest. This may also explain why colony V has a wider range of areas even under the control treatment (with the exception of one ant in colony VIII). In contrast, colony IV has a very large brood pile which is likely to impede the movement of the workers and therefore limit the change in space use between the two treatments resulting in a lower value for ϕ.

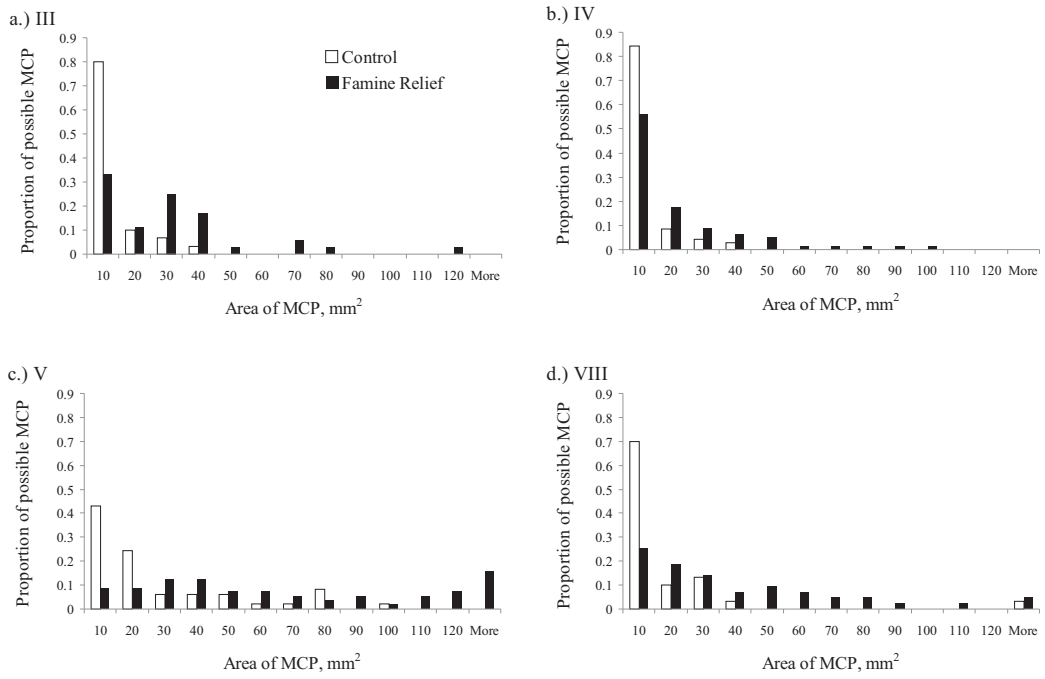

Figure 5-8: *Distributions of areas of the Median Convex Polygons, MCP, in each colony. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

5.2.3 Comparison of area available and area used per ant

Using the polygon of effective area we can calculate the area available per ant if they occupied this area with no overlap, i.e. dividing the effective area by the number of internal ants. Figure 5-9 compares the area available per ant with the average area used per ant taken from the areas of the individual MCPs. It shows that under the control condition each internal worker uses approximately the area that would be available per ant if the effective area inside the nest occupied by the colony were divided by the number of internal ants. This is consistent with a structured system where the individuals have spatial fidelity zones and there is minimal overlap in the areas they occupy. In contrast, under famine relief, the ants individually use a significantly larger area within the effective area which forces the area used per ant to be overlapped with the areas used by many other ants. By covering a larger area and overlapping with the space use of many of their nest-mates the ants potentially create a well mixed system within the area occupied inside the nest.

Previous studies have shown that workers of *T.albipennis* allow approximately 5mm^2 per ant when constructing nest walls, [92]. The solid bars in figure 5-9

show that the effective area allows between 5 and 10 mm^2 per internal ant under the control treatment in the four colonies. In comparison under the famine relief treatment the effective area allows between 5 and 13 mm^2 per internal ant. These areas are slightly larger than the 5 mm^2 per ant created when the ants are permitted to build the nest wall; this may be due to seasonal effects which cause increased activity and expansion of space use during the warmer seasons [10]. This slight increase in area during the famine relief treatment was apparent earlier in this chapter from figure 5-1 and from the total effective area shown in table 5.4. Colony V shows the greatest increase in effective area while colony IV shows the least change. The reason for this variation in change in effective area may again be related to the differences in geometry and demography between the four colonies. Colony IV's large brood may inhibit a larger effective area within the artificial nest whereas colony V has more space not occupied by brood items that the workers can expand into.

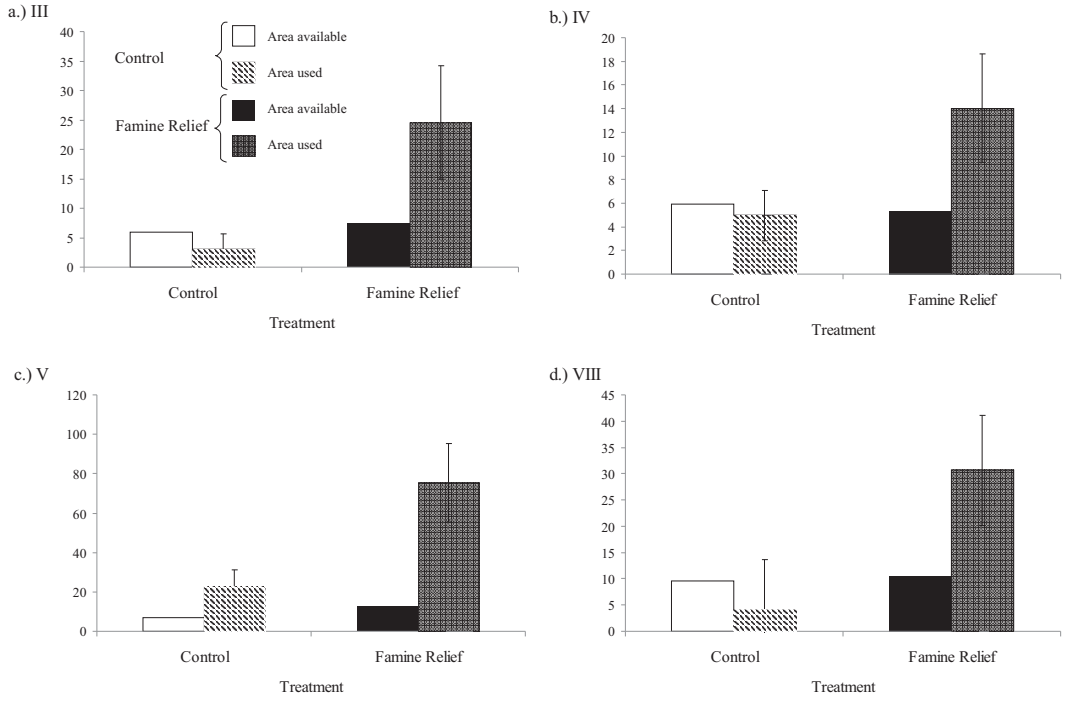

Figure 5-9: *Area available per internal ant, solid bars, and average area used per internal ant, striped bars, under the two treatments. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

5.2.4 Spatial overlap of internal ants

Figure 5-9 presents a representation of the amount of spatial overlap between individuals at the colony level. To explore in further detail the level of spatial overlap in the nest, we can look at the proportion of other ants' MCPs that an individual's MCP actually overlaps with.

Figure 5-10 shows that in all four colonies under control the proportion of other internal ants each internal ant overlaps with is very low in comparison to under the famine relief treatment. Colonies III and VIII are similar to one another under both treatments; showing a median spatial overlap of less than 0.1 under the control treatment and increasing to between 0.4 and 0.6 under the famine relief treatment. This similarity may be due to the similar colony size, however it is interesting that colony VIII has a slightly lower spatial overlap than colony III under the famine relief treatment given that this colony has more MCP's of larger area, see figure 5-8. Perhaps the larger, more scattered brood pile in colony VIII restricts the spatial overlap of internal workers more than the smaller brood pile in colony III. IV shows the least difference between control and famine relief, this is possibly due to the constraints in movement imposed by the relatively large brood pile. In contrast, colony V already has a relatively large overlap even under control compared to the other 3 colonies, then shows an even greater level of overlap under the famine relief treatment. However the amount of increase between the two treatments is similar to that in colonies III and VIII. Colony V has a much smaller brood pile further into the nest. This potentially allows the internal workers to move around more, shown by the bigger areas of MCP's in figure 5-8, thus creating more spatial overlap between the individuals. Colony V also had the fastest rate in the first feeding curves shown in Chapter 4, see figure 4-8 and table 4.8. The higher level of spatial overlap in this colony is likely to have facilitated faster food transmission.

5.3 Categorising the ants

In Chapter 6 I present analysis of the transmission pathways used during the famine relief treatment. Within this analysis it is useful to categorise ants by some aspect of their spatial behaviour, particularly as we know that their spatial position is related to the tasks they perform within the colony [107]. One way

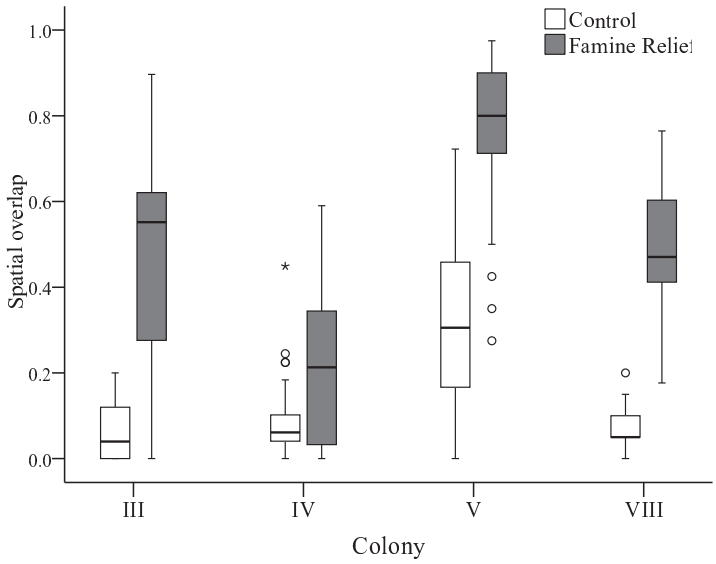

Figure 5-10: *Spatial overlap of internal ants defined as the proportion of other internal ant's MCPs that an internal ant's MCP overlaps with.*

to do this is to look at whether an individual was internal or external during the two treatments. If an ant was internal during a treatment they were inside the nest for all 31 SPS, while if an ant is classed as external for a treatment they were outside the nest for at least 1 SPS during that treatment. Ants that were internal for both treatments can be categorised as truly 'Internal', while ants which were external during both treatments as truly 'External', see figure 5-11. Ants that were internal during the control treatment and then became external during the famine relief treatment can be categorised as 'Recruits' (although they may not have actually been 'recruited' by another individual as in the traditional sense). Ants that were external during control and subsequently internal during the famine relief treatment can be categorised as 'Retreaters' (as in they 'retreat' back to the nest and stay there during the famine relief treatment). Figure 5-12 shows the proportions of ants in each of these categories in each colony. Interestingly there are only a small proportion of Recruits in each colony whereas there is a larger proportion of Retreaters in colonies III, IV and VIII. Chapter 6 will look at the role of these ants in particular why they don't leave the nest to forage during this time of need.

We have seen from figure 5-12 and figure 3-7 that there is a smaller proportion of external ants outside the nest during the famine relief treatment compared to during the control. This is interesting as it shows there are less ants foraging during the famine relief treatment. Figure 5-13 shows that in three out of four of

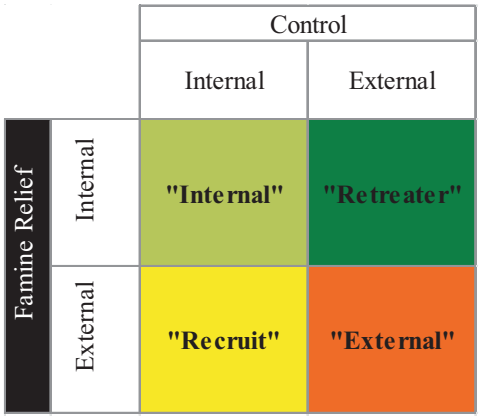

Figure 5-11: *Table showing the category individuals are placed in based on whether they were internal or external in the two treatments.*

the colonies the smaller number of external ants under the famine relief treatment undertake a higher number of trips outside the nest than the external ants in the control treatment. This shows that instead of increasing the number of foragers, individuals increase their foraging effort during famine relief. Colony IV does not show this increase so may use a different method to increase the food distribution, we will see in Chapter 6 that two of the foragers in this colony feed a very large proportion of the colony. These two foragers are shown by the out-liers for colony IV under the famine relief treatment making 7 and 8 trips outside the nest within the 30 minutes.

As well as the relatively simple method of categorising the ants shown in figures 5-11 and 5-12 we can use the SPS data to make a more fine scale classification. It appears that most of the MCPs fit into one of four zones; the area containing the brood, the area immediately surrounding the brood, the area between the brood and the nest entrance, and the remaining outer areas of the nest. We can use these zones to categorise each individual. Figure 5-14 shows an outline of these zones for each colony. The 'Brood' zone is formed by creating a convex polygon around the coordinates of the brood items. The 'Ring' zone is then formed by expanding the points on the Brood polygon out by 4mm (2 bodylengths) from the centre of mass of the brood pile. The 'Arena' zone is taken as the area between the nest entrance and the brood and is the width of the widest part of the Brood polygon. The edges are formed by projecting this widest part onto the entrance wall of the nest along the line between the centre of brood pile and the centre of the nest entrance. Each ant was then categorised based on which zone they had the highest number of SPS in. Figure 5-15 shows the proportion of internal and external ants in these zones during the two treatments. It highlights the shift

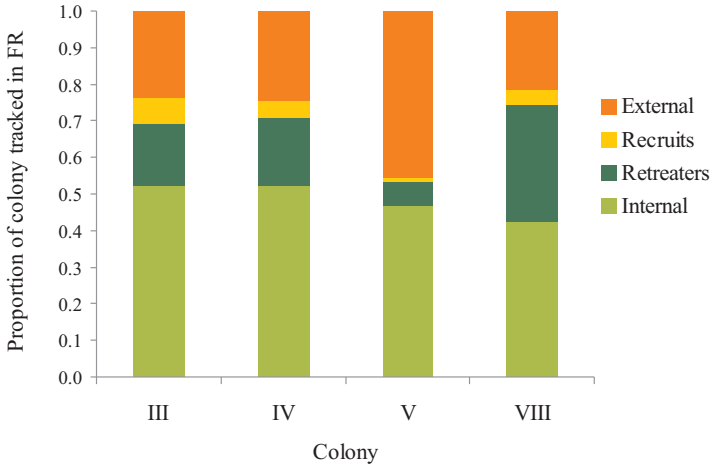

Figure 5-12: *Proportions of External, Internal, Recruits and Retreaters in each colony. External ants went outside the nest both treatments; Recruits were inside for control and went outside during famine relief; Retreaters went outside during control but remained inside the nest during famine relief; Internal ants remained inside the nest for both treatments.*

away from the brood and into the arena by the internal ants between the control and famine relief treatments. These classifications will be used in Chapter 6 to investigate the amount of food transmitted to individuals in these zones.

5.4 Final spatial distribution of food inside the nest

The traditional method commonly used to track food distribution inside the nest of a colony uses radio-active isotopes. While our method provides much finer detail at the individual level we can also provide the colony level information that traditional methods are capable of by combining the information from the SPS and the trophallaxis data. As described in Chapter 2 we can use the duration of the feeding events as a proxy for how much food has been transferred from the donor to the recipient. Chapter 6 goes into more detail as to how we calculate this.

Figure 5-16 shows the final destination of food in each of the four colonies in relation to the brood pile and nest outline. The figure shows that in colony III much of the food ends up close to the brood pile. In colony IV there are two

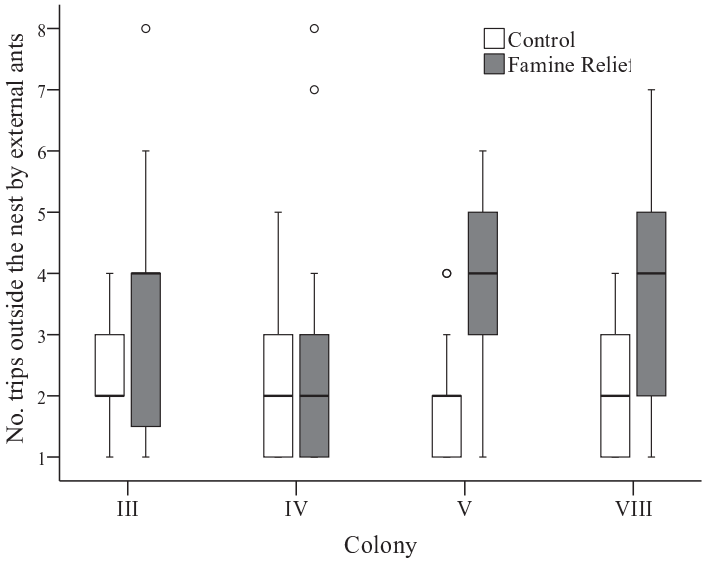

Figure 5-13: *Number of trips outside the nest by external ants during both treatments.*

clusters of large amounts of food, one in the arena and one above the top left corner of the brood. Meanwhile colonies V and VIII appear to have food dispersed over a wider area of the nest. This is a similar result to that seen in [78] where scintigraphy was used to monitor the flow of food inside a nest of *Formica fusca*. However due to the relative simplicity of the nests used in this project we are able to give additional details such as the location of the brood and the distribution of background food, shown by the green circles in figure 5-16, as well as the newly introduced food.

5.5 Summary

This chapter has addressed objective B: to determine whether the spatial structure inside the nest is maintained during the famine relief treatment. Several features of the space use of the workers have revealed a distinct change between the treatments showing that the spatial structure is abandoned during famine relief in a way which facilitates the rapid transmission of food. We have seen that the effective area used by the workers is much smaller than the 1248 mm^2 available inside the artificial nests, figure 5-7. There were two hot-spots of space use by workers during the control treatment: one on the brood pile and one close

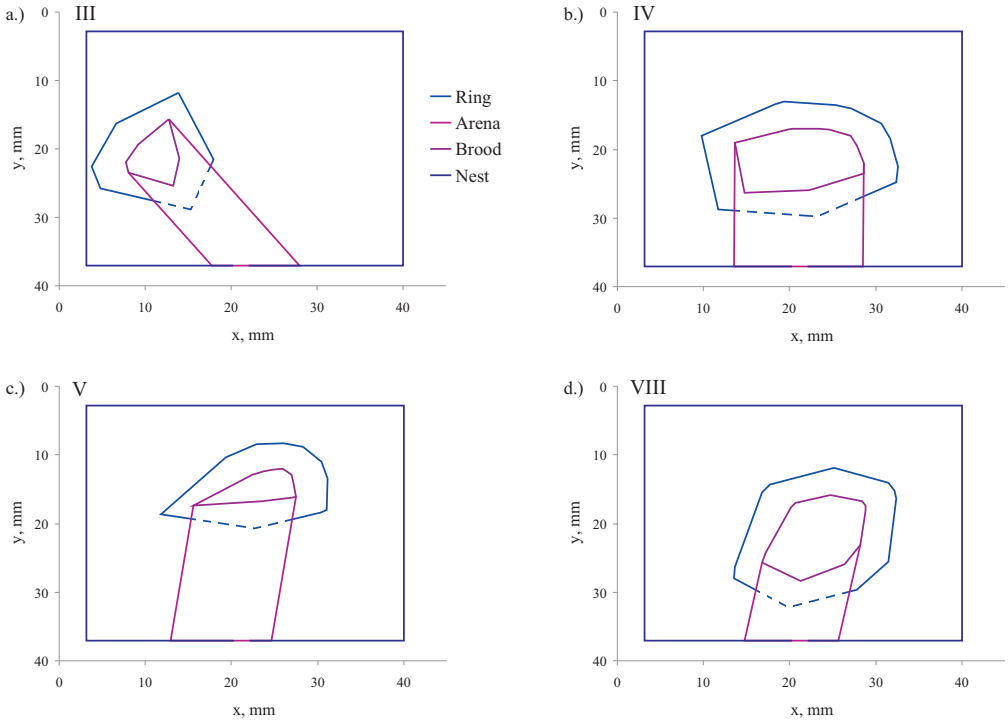

Figure 5-14: *Zones used to categorise ants, Brood, Arena, Ring, Nest, Outside. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

to the nest entrance in each colony, fig. 5-1. These are consistent with the spatial structure identified in [95] and [107]. During the famine relief treatment these hotspots are less concentrated and shift away from the brood and into the arena between the edge of the brood and the nest entrance. The internal and external ants become closer together during the famine relief treatment, figure 5-2, facilitating the transmission of food from the external to the internal workers. Both the internal and external ants move closer to the brood pile as a function of time during the famine relief treatment in three out of four of the colonies, figure 5-4. At the start of the famine relief treatment the centre of mass of the ants in these colonies was further from the brood pile than during the control. The shift towards the brood during the famine relief treatment is likely to be the ants returning to their usual structure once the food distribution is well under-way. Previous studies in this species have shown that workers re-adopt their spatial positions inside the nest after an emigration, known as 'social resilience' [108]. Further analysis of individual space use beyond the thirty minutes is required to determine whether this occurs after famine relief is complete but it appears that brood workers are beginning to return to their positions by the end of the treatment. Meanwhile the external ants may be moving deeper into the nest to feed remaining unfed workers which are likely to be further from the nest entrance. In

colony IV this trend is reversed: workers begin the famine relief treatment closer to the brood centre compared with under the control treatment and subsequently move further away. This is perhaps a consequence of the relatively large brood pile in colony IV compared to the other colonies which is likely to restrict the movement of ants around the brood. As well as being closer to the external ants the internal ants become potentially more mixed under famine relief shown by an increase in spatial overlap with one another, figures 5-9 and 5-10, and in the area they cover, figures 5-7 and 5-8. These changes in space use all demonstrate that during the famine relief process the ants are loosening their spatial structure and potentially becoming mixed to facilitate the food distribution.

A study in *Formica fusca* revealed that the centre of gravity of food inside the nest stabilizes as a function of time and its final location is in the centre of the nest [78]. Figure 5-16 shows the final locations of the food in all four colonies. It shows that in colonies III and IV the food is concentrated around the edges of the brood pile while in V and VIII it is much more scattered throughout the area of the nest. Given that we expect the ants to eventually return to the spatial organisation seen under the control treatment once famine relief is complete we might expect the food in V and VIII to gravitate towards the brood pile as in III and IV. If we look back at the differences in space use between the two treatments, for example figures 5-6 and 5-7, we see that III and IV showed less individual and colony level expansion than V and VIII. This may explain why their final locations of the food are closer to the brood and why V and VIII are more widespread. Further analysis beyond the 30 minutes of the famine relief treatment would be required to determine whether the food in colonies V and VIII eventually becomes concentrated around the brood pile.

The spatial position of workers in *T. albipennis* relates to the tasks they perform, [95, 107], and therefore may influence the amount of food an individual receives and how she receives it, for example, directly or indirectly from a forager. In this chapter workers were firstly categorised by whether they were internal or external during both treatments and if they changed from being internal to external, 'recruits', or vice versa, 'retreaters', see figure 5-12. This revealed that, in addition to a higher proportion of ants inside the nest during famine relief compared to under control, there are also very few recruits. This is a surprising result given that species such as *T.albipennis* which scavenge for their food are expected to recruit many nest-mates to a food source due to competition and the unpredictable nature of their environment [111]. As the food source is close to

the nest entrance ($\approx$ 1cm) and there is no competition, perhaps it is more efficient to keep these workers, the 'retreaters', inside the nest. This allows them to re-distribute the food to other workers so the existing foragers are able to return to the food source more rapidly. The foragers therefore increased their individual foraging effort as opposed to increasing the number of foragers through recruitment during famine relief. This has also been seen in honey bees [65]. The ants can also be categorised by their space use within the nest using defined zones, see figures 5-14 and 5-15, similar to those defined in a previous study [107]. These classifications will be used in Chapter 6 while looking at the amounts of food individuals receive (Objective C) and through which pathways (Objective D).

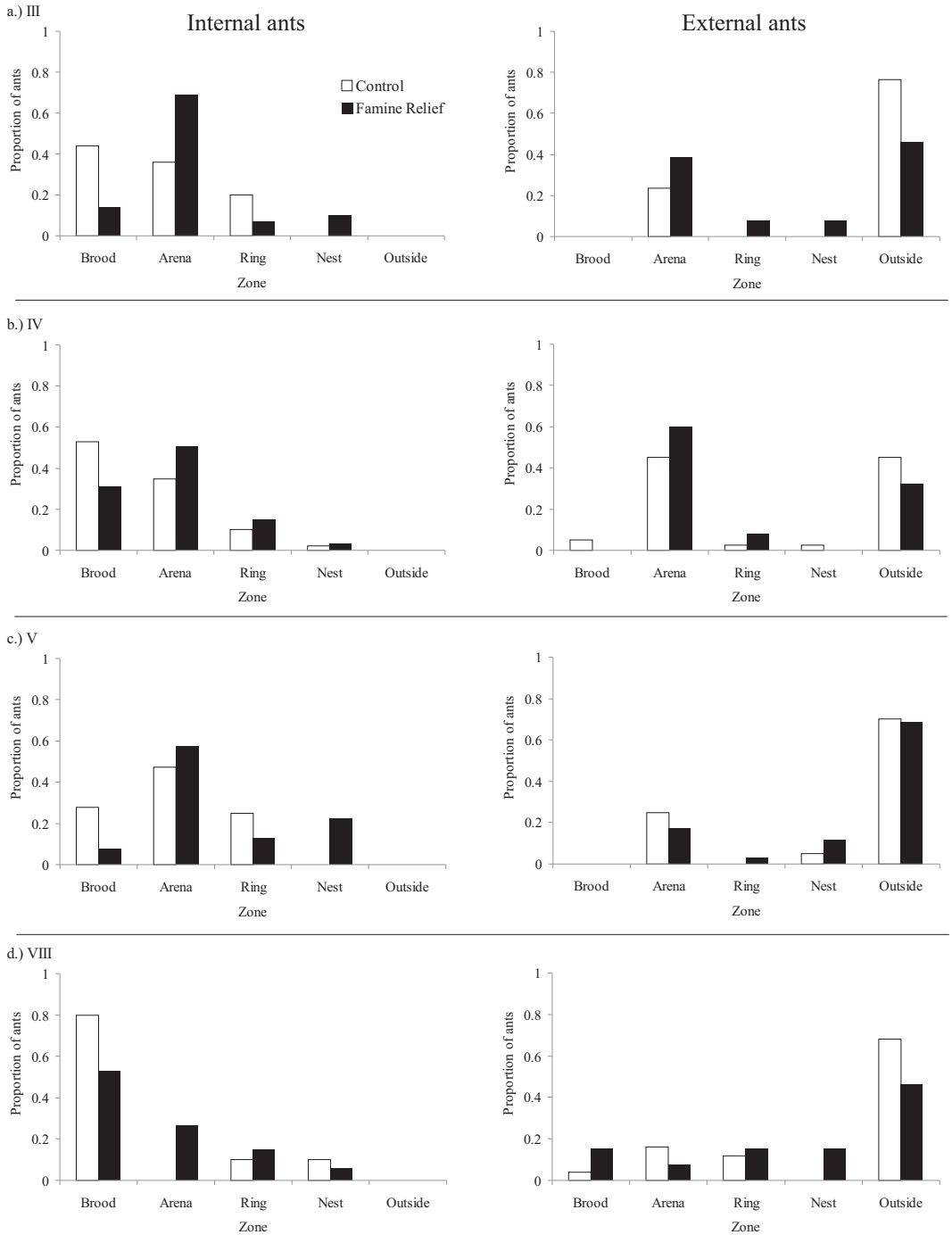

Figure 5-15: *Proportions of ants in the five spatial zones under the two treatments. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

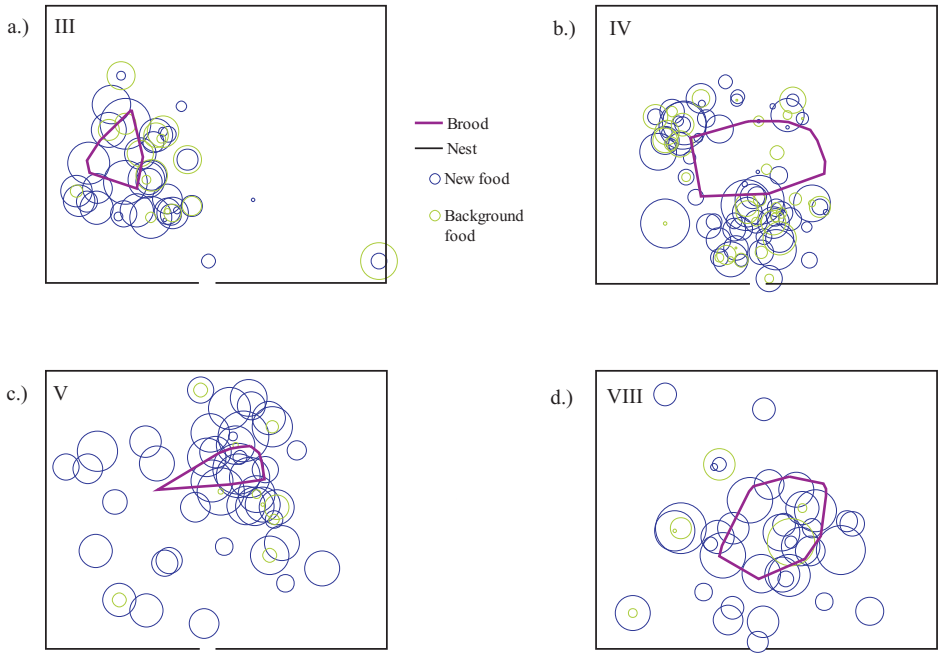

Figure 5-16: *Final destination of food inside the nest under famine relief for each colony, locations of circles represent an ants location at* $t = 1800$ *seconds, i.e. the 31st SPS for an ant with a non-zero amount of food. The area of the blue circles represents the amount of "new food" an ant at that location has while the area of the green circles represents the amount of "background" food an ant at that location has. An ant may contain new and background food. a.) Colony III,* N_c*=42; b.) IV,* N_c*=95; c.) V,* N_c*=77; d.) VIII,* N_c*=49.*

Chapter 6

Amounts of food and transmission pathways

This chapter addresses objective C: to determine the amounts of food distributed to individuals, establish whether the food is evenly distributed among workers, and whether the colony capacity is reached; and objective D: to deduce the structure of the transmission pathways used to distribute food among workers and determine if there are preferences for who feeds whom. If individuals do not all receive an equal amount of food we may expect the amount they receive to be related to the role they perform within the colony and therefore the spatial class of individuals as identified in Chapter 5 will be used to investigate this aspect.

A feature that could not have been predicted at the outset of this project was the distribution of stored food in parallel with the newly introduced food. This food has been physically stored inside the crops of workers for the two day starvation period. (An ant's 'crop' is a reservoir for the retention of liquid food which is connected to but separate from their stomach where food is eventually digested.) These workers are still alive, apparently unharmed from the food and able to pass it on to their nestmates in so-called 'background feeding events'. This implies that the stored food was not harmful to them and is relatively safe for distribution within the colony. This chapter looks at which individuals provide and receive this background food and uncovers a potential mechanism that triggers individuals to donate their stored food. Storing food may serve two functions; firstly, to test the toxicity of the food and secondly to provide a reserve supply for times of extreme food shortage. In contrast, the food introduced in the dish outside the nest at the

start of the famine relief treatment is 'new', untested and could contain pathogens which the foragers might not be able to detect [55]. In addition to this, during the famine relief treatment a large amount of food is introduced to each colony in a short period of time via many interactions between individuals. This potentially increases the colony's vulnerability to any pathogen or parasite that may be in the newly introduced food. We might therefore expect the colonies to structure their transmission networks and organise their feeding behaviour in a way that provides protection. Examples of how colonies might do this include feeding brood carers less, or partitioning the transmission networks for each forager, see as an example figure 6-1. In this way the ants in each pathway are exposed to only one forager thus partitioning the potential risk. An alternative is that the ants aim to mix which foragers they are exposed to as much as possible to promote the spread of social immunity and possibly reduce the toxicity of a poison that one forager may have introduced by diluting it with food from other foragers.

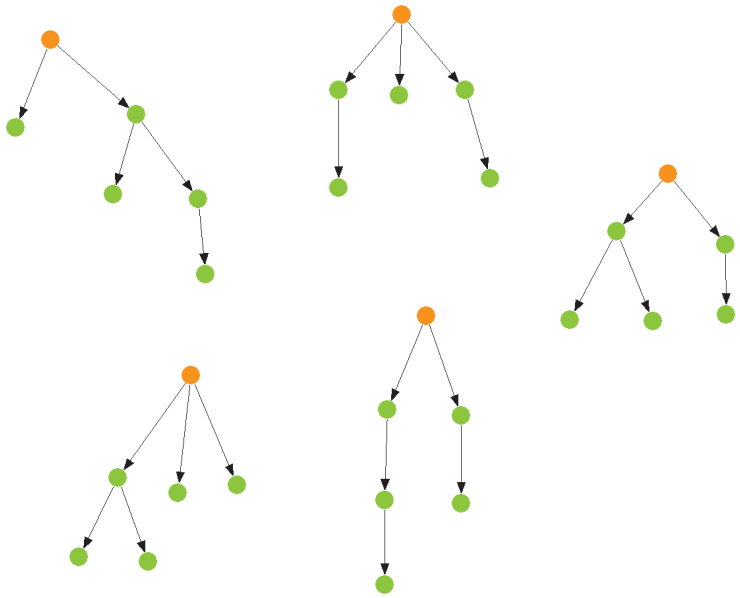

Figure 6-1: *An example of a partitioned transmission network where each internal ant (green nodes) is only exposed to one forager (orange nodes).*

The features covered in this chapter include: the total amounts of new and background food provided in each colony and to each individual investigating whether individual role is related to the amount received; the structure of transmission pathways used to distribute new food under the two treatments; further analysis of these pathways during famine relief to determine whether the networks are partitioned or mixed in the context of how a pathogen might spread; and finally an exploration of the 'background feeding' phenomenon. Before the more detailed

analysis of the amounts and pathways of food transmission the first half of this chapter describes the data refinement that was needed in order to calculate the amounts of food transmitted.

6.1 Data refinement

Studies which look at the transmission of food within social insect colonies typically use the method of tracing a radio-active isotope, for example [73, 78]. In this experiment, as the food is not labeled with any traceable marker, it is not possible to use the same techniques to quantify the amounts of food transferred. However, there is evidence to suggest that the duration of a trophallactic interaction is related to the quantity of food transferred [117, 37]. The careful tracking and observation of each ant in this experiment means that the duration of each trophallactic event is available and can be used to estimate the amount of food that was transferred during each event.

Before the calculation can be performed, one point needs to be considered. Many of the trophallactic events during the famine relief treatment involve multiple recipients feeding simultaneously in a rosette from a single donor. In order to calculate a proxy for how much food has been transferred from the donor to each individual we need to determine whether the rate of transfer with multiple recipients is driven by the donor or the recipients. In other words, when there is more than one ant receiving from one donor, does the donor pump food out at the same rate as she would if there was only one ant receiving from her, thus meaning that each unit of food received needs to be divided by the number of recipients simultaneously receiving from that donor. This hypothesis will be referred to as H_{donor}. Alternatively, does the donor increase the rate at which she pumps food out to compensate for the fact that there are more ants receiving food from her, meaning that the amount each recipient receives is directly proportional to the duration that she was receiving for. This hypothesis will be referred to as $H_{\text{recipient}}$. It could also be the case that the donor pumps food out slower when there are more recipients if somehow the recipients crowding around her makes the process less efficient compared with feeding only one recipient at a time. To determine which hypothesis is more accurate the durations spent drinking at the honey solution, DR, (see section 2.3.4) need to be compared with the durations of

subsequent donations which are extracted from the trophallaxis data (see section 2.3.2).

There are two different ways of considering the duration of a donation when there is more than one recipient. Firstly, the 'Donor Duration', DD, is the overall length of time that the donor is donating for. Secondly, when there is more than one recipient receiving from a donor simultaneously it is possible to sum the individual durations that each recipient is receiving for giving a 'Total Recipient Duration', TRD. The fraction DR/DD gives the number of units drank for each unit donated, e.g. if an ant drinks for 10 seconds and donates for 5 seconds she drinks 2 units for every one donated. To verify which hypothesis is more accurate I will look at the relationship between the fraction DR/DD and the number of recipients in the corresponding donating event. The average number of recipients, N_r, is used because recipients join and leave a rosette at different times. N_r is calculated by taking the sum of the number of recipients each time-step (i.e. every second) over all time-steps for the donation and dividing by the total number of time-steps the donor was donating for:

$$N_r = \frac{\sum_{t=a}^{b} n_r(t)}{\mathrm{DD}} \tag{6.1}$$

where a is the start time of the donation event, b is the end time and $n_r(t)$ is the exact number of recipients receiving from the donor at time t. This value is related to the total recipient duration, TRD, as follows:

$$N_r = \frac{\mathrm{TRD}}{\mathrm{DD}} \tag{6.2}$$

and therefore it is not necessary to investigate the relationship between the fraction DR/TRD and N_r. If $\mathrm{H_{donor}}$ were true then the rate at which food is pumped out would be independent of N_r so DR/DD would be constant regardless of the number of recipients as shown in figure 6-2 a.). In this case the donor regurgitates one unit of food per time step regardless of how many recipients are feeding from her at the same time and the amount each recipient receives should be divided by the number of recipients latched on at each time step.

If $\mathrm{H_{recipient}}$ were true then the rate the food is pumped out would increase with N_r as shown in figure 6-2 b.). In this case the amount each recipient receives should be the duration that each recipient receives for without dividing by the number of recipients.

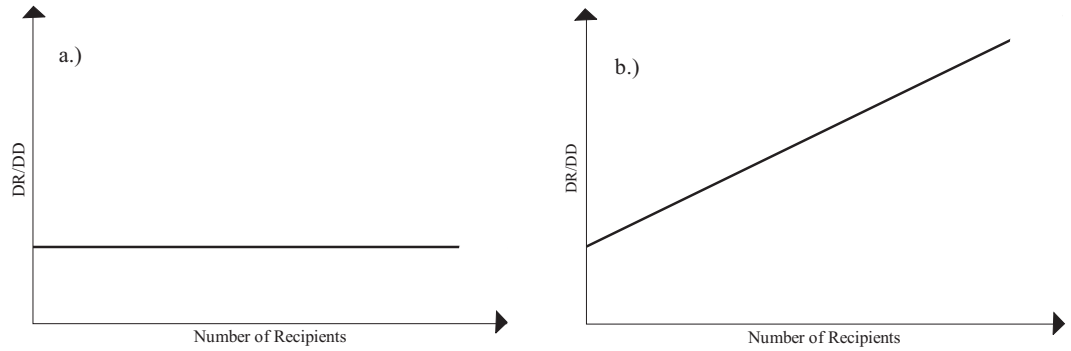

Figure 6-2: *Predicted relationships between average number of recipients, N_r, and the rate at which the amount drunk at the honey solution is donated. a.) DR/DD for H_{donor} where rate is independent of N_r. b.) DR/DD for $H_{recipient}$ where the rate increases with N_r.*

6.1.1 Complete round trips

To investigate the relationship between the fraction DR/DD and the average number of recipients, N_r, we need to consider the relevant data from 'Complete Round Trips', CRTs carried out by foraging ants. A CRT consists of a sequence of activities by a forager starting with a drinking event outside the nest, returning to the nest and donating, and finishing with the ant leaving the nest and drinking again or leaving the nest without returning for sufficient amount of time to deem the ant empty. The assumption made here is that if an individual still possessed food to donate in her crop she would remain inside the nest, i.e. foragers only leave the nest when they are empty. I used the spatial data collected for each ant in section 2.3.1 along with the trophallaxis data and drinking data to find the CRTs for the foragers.

As described in Chapter 2, the identity of ants drinking at the honey solution outside the nest were recorded on the audio channel (AC) for each colony under both treatments. For each time an ant drank at the honey solution their ID, start time and end time of the drinking bout were recorded from which the duration is calculated. Often the data for any one forager was insufficient to include as a CRT, e.g. the end time of a drinking event might not have been recorded, or the ant donated inside the nest and then did not leave the nest again so it was not possible to determine whether this ant had emptied her crop or not. In addition to this, because the number of recipients is important a round trip where the forager donated in two different rosettes or one rosette and one pairwise interaction could not be used, however if the forager donated in multiple pairwise

interactions the trip could be used. Because there were not many usable CRTs per colony, see table 6.1, I accumulated the data for all four colonies.

Colony	**III**	**IV**	**V**	**VIII**
Number of trips Control	2	2	1	1
Number of trips Famine Relief	6	10	7	12

Table 6.1: *Number of identifiable and useable complete round trips per colony*

6.1.2 Verifying the proxy for the amount of food

Using the data from the CRTs I carried out a regression analysis to look for a relationship between DR/DD and the average number of recipients, N_r, shown in table 6.2 and figure 6-3. The gradient was not significantly different from zero at the $\alpha = 0.05$ significance level. This is consistent with the prediction for the H_{donors} hypothesis that a donor donates food at the same rate independent of the number of recipients, i.e. donors pump out one unit of food per time-step.

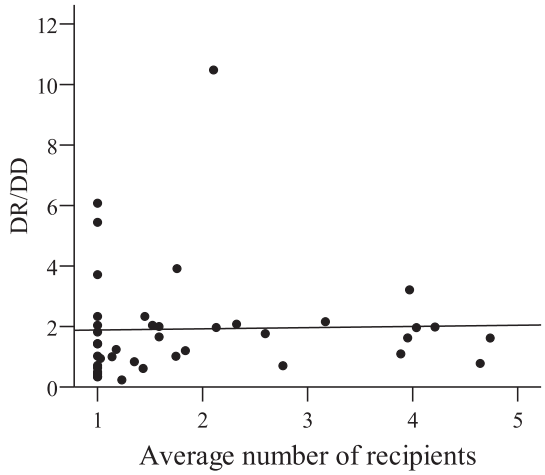

Figure 6-3: *DR/DD against average number of recipients. Line shows linear fit.*

6.1.3 Implementing the proxy for food

Now that it is known that the amount of food donated is driven by the donor and is independent of the number of recipients receiving simultaneously a net-food

R^2	F	df1	df2	Sig.	Constant	Gradient
0.001	0.0025	1	39	0.876	1.845	0.039

Table 6.2: *Linear regression analysis on DR/DD against average number of recipients, N_r, with N_r as the independent variable*

time-line for each ant can be constructed, see figures 6-4 and 6-5. These can be calculated from the time-lines introduced at the end of Chapter 2, see figures 2-11 and 2-12. The receptions need to be adjusted by taking each time-step an individual is receiving for and dividing the one unit received by the number of ants receiving from the same donor in that time-step. The net-food is then calculated each time-step by summing the donation and reception time-lines for that ant. An additional adjustment implemented in the net-food time-lines is to add on the amount drank at the honey solution by each forager at the time they return to the nest, using the entrance lists. As mentioned previously we do not know the drinking times for all the foragers, therefore the amount added at the time they enter the nest is equal to the amount they subsequently donate while inside the nest. For example see figure 6-5 a.) which shows that the ant enters the nest at times $t = 450$, $t = 850$ and $t = 1420$, these now correspond to an increase in net-food in part d.) the net-food time-line. The amount of increase at these times is equal to the amount the ant donates before she leaves the nest again. The dashed line in figure 6-5 d.) shows the net-food for this ant before this adjustment was made and illustrates how the net-food would become negative for a forager without this adjustment. These adjustments were made using algorithms written in Fortran.

6.1.4 Separation of background food

On examining the refined net-food time-lines for the famine relief treatment a further issue became apparent. There were several internal ants, i.e. not foragers, in all four colonies whose net-food time-lines went negative by a large amount. I revisited the videos to verify that these individuals were not foragers. Occasionally an individual would go negative by a small amount, consistent with small errors in the recorded timings of the feeding events. However, a final net-food of minus 300 units, which is large relative to the distribution of final net-food amounts, see figure 6-7, cannot be explained by such errors.

To end up with a large negative net-food means that the individual had donated a large amount of food while receiving much less within the thirty minutes. These

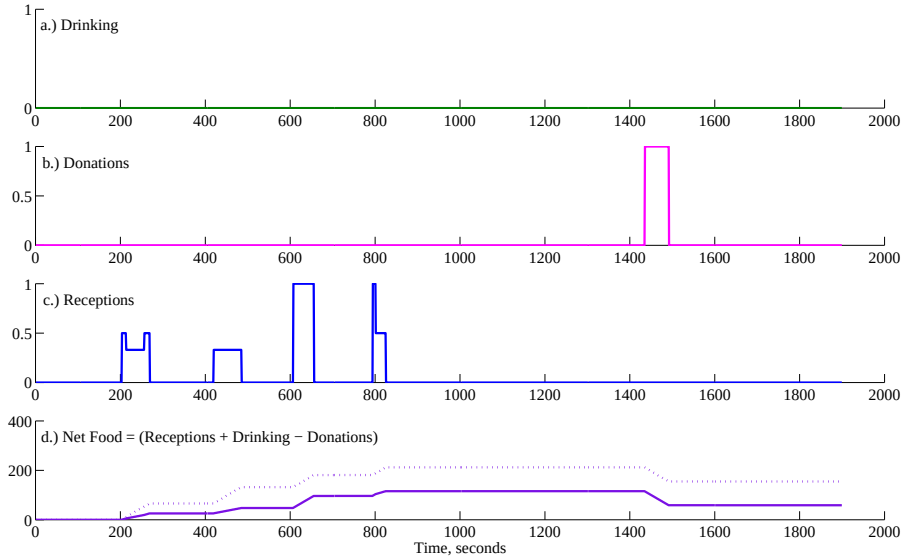

Figure 6-4: *An example of a set of adjusted timelines for an internal ant. This is ant 22 from colony VIII. a.) Drinking, b.) Donations, c.) Receptions, d.) Net-food calculated by the cumulative sum of Receptions + Drinking - Donations. Dashed line represents original data prior to the adjustments. This ant does not go outside the nest therefore there is no drinking.*

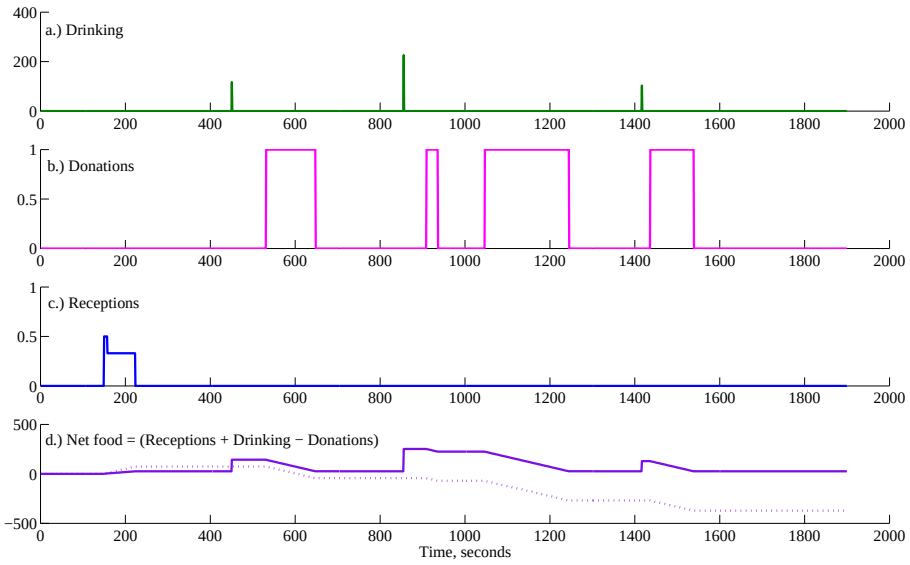

Figure 6-5: *An example of a set of adjusted timelines for an external ant. This is ant 6 from colony VIII. a.) Drinking, b.) Donations, c.) Receptions, d.) Net-food calculated by the cumulative sum of Receptions + Drinking - Donations. Dashed line represents original data prior to the adjustments. As seen in figure 2-12, this ant re-enters the nest 3 times relating to the three drinking events seen in a.).*

individuals must have been donating food that they had stored in their crops during the control treatment previously. This food is what I have referred to as 'Background food' and must have been stored for at least two days (the period between the control and famine relief treatments). As mentioned previously in this chapter this length of time would be long enough for the individuals to test the toxicity of the food and therefore this food can be considered different from the newly introduced honey solution. These large donors of background food and what triggers them to donate will be explored at the end of this chapter.

I further refined the data to separate the background feeding from the newly introduced honey solution feeding so that the two could be analysed separately. I wrote an algorithm in Fortran that separated the background feeding into a separate time-line any time an individual's net-food time-line went below zero. This algorithm worked causally so that any background feeding donated towards the beginning of the 30 minutes and subsequently passed on later would be detected and corrected for. For the control treatment there was no need to separate out the background feeding as there was no distinction between 'old' and 'new' food as the food was provided continuously.

6.1.5 Exposure matrix

Later in this chapter I will explore how much the ants are exposed to foragers during the famine relief treatment in the context of managing the risk of a pathogen spreading with the food. Using the information from the time-lines described above I have constructed an $N_c \times N_c \times t$ matrix, $\mathbf{E}[N_c, N_c, t]$, which represents the cumulative amount that each ant is exposed to new food from every other ant. N_c is the number of ants in the colony and t is time in seconds ranging from 0 to 1800. The entries of this matrix are calculated causally, assuming that at $t = 0$ all entries are 0. Each subsequent entry, $\mathbf{E}[j, i, t]$, is then calculated by adding the amount of new food, c, donated in time-step t by ant j to ant i, to the $\mathbf{E}[j, i, t-1]$ entry:

$$\mathbf{E}[j, i, t] = \mathbf{E}[j, i, t-1] + c. \tag{6.3}$$

The information about which individual is the donor is stored in the 'Donor' time-line, see section 2.5, so the direct and indirect exposure can still be distinguished.

To include the indirect exposure to ant i the $\mathbf{E}[x, j, t-1]$ entry for $x = 1, N_c$; $x \neq i$; is added to $\mathbf{E}[x, i, t-1]$ provided it is less than c, if it is greater than c then the amount c is added to the $\mathbf{E}[x, i, t-1]$ entry:

$$\mathbf{Exp}[x, i, t] = \begin{cases} \mathbf{E}[x, i, t-1] + \mathbf{E}[x, j, t-1] & \text{if } c > \mathbf{E}[x, j, t-1] \\ \mathbf{E}[x, i, t-1] + c & \text{if } c \leq \mathbf{E}[x, j, t-1] \end{cases}$$

Calculating the exposure this way makes sure that it is causal and that no ant is calculated as being exposed indirectly to more from an ant than it has been directly exposed to from the direct donor. Causality is important when looking at the transmission of an actual entity such as food in this case because the order in which events occur affect whether an ant is exposed to another one. This effect will be explained in more detail later in section 6.4.

6.2 Amount of food analysis

Now that the data have been refined to calculate the correct amounts of food and the background food filtered into a separate time-line for each ant we can begin to address the amounts of food transmitted during the famine relief treatment.

Figure 6-6 a.) shows the total amount of food provided during the famine relief treatment in each colony. This total is made up of the new food brought in by the foragers and the background food provided by ants which were internal during the famine relief treatment. It shows that colonies V and VIII provided the most food in total while a bigger proportion of the food provided in colonies IV and III was background food compared with V and VIII. Part b.) shows the amount of food provided per capita, i.e. dividing the total amount of food by the number of ants in the colony, N_c, taken from table 4.5. This shows that per capita the two smaller colonies, III and VIII, actually provided more food, while colony IV provided the least. Part c.) shows the average net-food per 'fed ant', this was calculated averaging the final net-food, i.e. at $t = 1800$ seconds, of all ants that had non-zero net-food for the respective food type. This omits foragers who generally have a zero net-food (unless they actually received food from trophallaxis inside the nest), we can assume that foragers would provide food for themselves at some point while drinking at the honey solution however

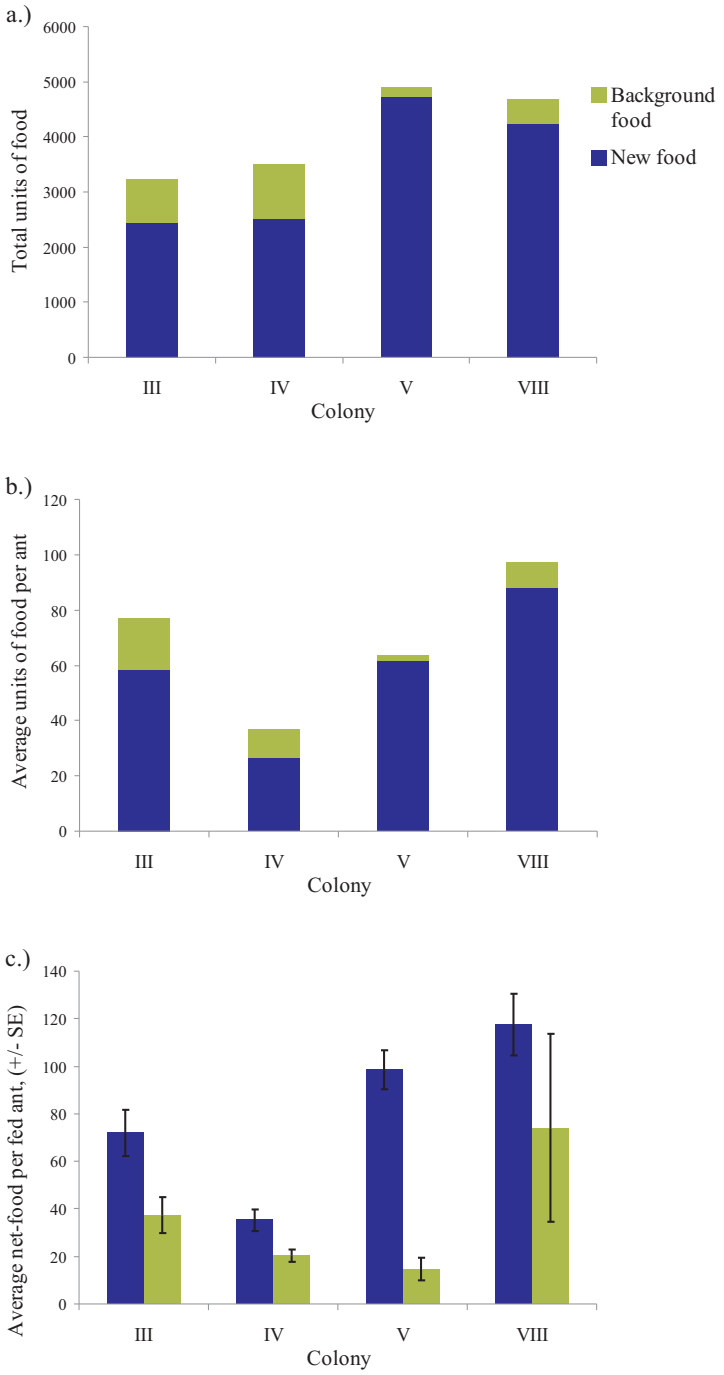

Figure 6-6: *a.) The total amount of new and background food transferred during the famine relief treatment, b.) the amount of food transferred per ant, and c.) the average net amount received per fed ant, in all four colonies.*

it is not possible to determine how much. This analysis shows that colony VIII provides the largest amount of food per fed ant. A possible reason for colony VIII to be providing such a large amount of food during the famine relief treatment compared to the other colonies becomes apparent when we compare the total amounts provided during the control treatment: III $\approx$ 780 units, IV $\approx$ 910 units, V $\approx$ 950 units and VIII $\approx$ 240 units. Colony VIII is providing a much smaller amount during the control treatment, this suggests that the colony is potentially more hungry at the start of the famine relief treatment. While the presence of background feeding in this colony indicates that a small number of ants did have stored food, (see fig. 6-30 later), perhaps none was donated during the starvation period resulting in the other workers being very hungry and requiring more food than the other colonies. Interestingly colony V still distributes the food to its members more quickly, see figure 4-8, which may indicate that hunger levels were also high in this colony compared to those in colonies III and IV.

Figure 6-7 shows the distributions of the final net-food for all ants in all four colonies at the end of the famine relief treatment. In this case the proportion of ants in the 0 net-food column is higher than the 5% that are unfed at the end of the famine relief treatment, see table 4.5. This is because this column also includes foragers which did not receive food inside the nest (but did donate) for example ant 6 in colony VIII shown in figure 6-5. The distributions show long tails for the new food. A small number of individuals in each colony receive a relatively large amount of food, i.e. over 200 units. In their study Wilson and Eisner showed similar distributions for the several different species of ant they investigated, although it appears that the individuals with the large amount of food in their case were the foragers that were bringing the food into the nest [73]. Due to the technique used they were not able to do some of the more fine-scale analysis that follows in this chapter, such as the transmission networks, or the separation (or even existence) of background food. Colony VIII has the longest tail with the most ants receiving a large amount compared to the other three colonies. In contrast colony IV has most ants receiving less than 50 units of food, perhaps in this large colony it is more difficult to get a large amount of food round to lots of ants.

It is clear from figure 6-7 that some individuals receive more food than others. In Chapter 5 the ants were classified based on the zone in which they spent the most time during the control treatment, see figure 5-15. Using this classification we can look to see whether the individuals that receive the largest amounts of

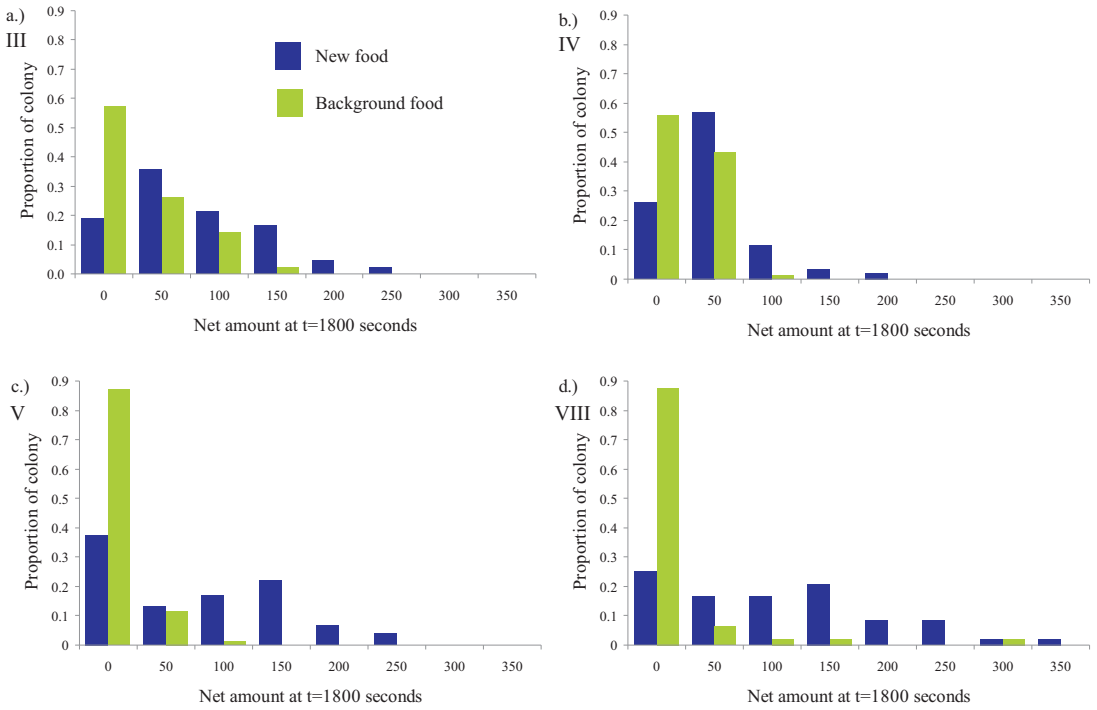

Figure 6-7: *Distributions of net amount of new food and background food at* $t = 1800$ *seconds during the famine relief treatment. a.) Colony III,* N_c*=42; b.) IV,* N_c*=95; c.) V,* N_c*=77; d.) VIII,* N_c*=49.*

new food belong to a particular task group determined by their spatial class. Figure 6-8 shows that the median net amount of new food of the brood ants is higher than for other spatial groups in colonies III, V and VIII. The individuals which have the largest net amount of new food tend to be brood ants and ants which were in the arena during the control treatment with a few external ants in colonies IV and VIII also having a large net-amount.

We saw in section 4.10 that between 63 and 76% of the fed ants in each colony received food of either type more than once meaning that the total amount received can be split into that received during a first feeding event and that received during subsequent feeding events. Figure 6-9 a.) shows that more food in total is received during subsequent feedings as opposed to first feedings. However part b.) shows that the amounts transmitted per event are similar between first feedings and subsequent feedings (there are a greater number of subsequent feeding events). This shows that the ants are receiving food in multiple events adding up to a large total, but the amounts received per event are similar regardless of whether it is a first feeding event or subsequent feeding event. There may be some risk management being under-taken by receiving food in multiple small

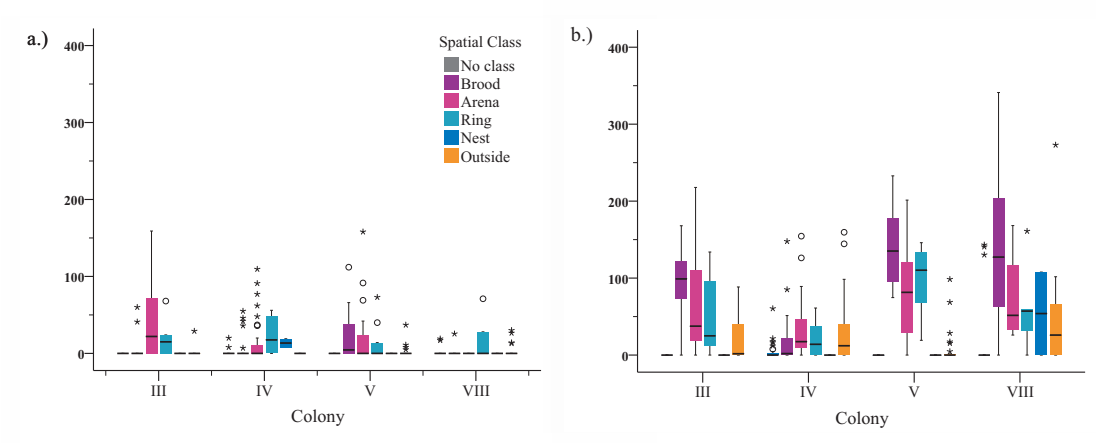

Figure 6-8: *Net amount of food received by individuals at the end of the 30 minutes of: a.) the control treatment, b.) the famine relief treatment, separated by spatial zone most used during the control treatment.*

amounts as doing so might dilute the effect of one bad batch of food. In addition receiving in multiple smaller events may be a faster way to get food round to all the ants at the start of the famine relief treatment compared with distributing it in larger amounts.

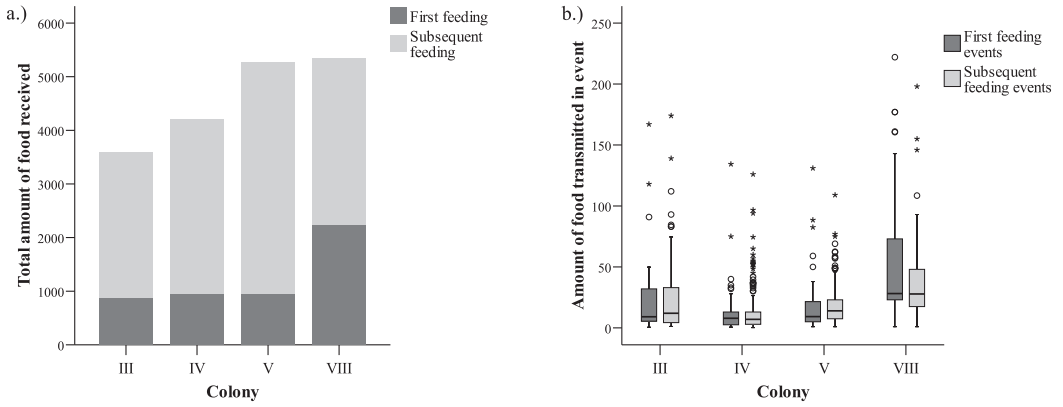

Figure 6-9: *a.) Total amount of food received during the famine relief treatment, b.) amount of food transmitted per event. (Note total values are larger than those in figure 6-6 a.) because this is amount received not amount provided).*

Using the spatial zones defined in section 5.3 we can look at the amounts of new food transmitted under the famine relief treatment to the various spatial classes. For this I have used the zone each ant used the most under the control treatment as this represents their typical space use. Figure 6-10 a.) shows how much food went to each of these zones, it shows that in colonies IV and V a large amount of food went to ants classed in the arena while in III the food was split between

the brood ants and the arena and in VIII most of the food went to the brood ants. However, this representation does not take into account the number of ants in each of these classes. Figure 6-10 b.) shows the proportion of the capacity of each class reached. The capacity of a class was calculated by multiplying the number of ants in a class by the largest net-food of any ant in that colony. The largest net-food of an ant in a colony shows the potential volume of food that an ant can hold and given this is a monogynous species we can assume that all the workers in a colony can potentially hold the same volume. There is evidence that foragers can hold more because they are stripped of lipids at the start of spring [23]; however this study was performed towards the start of winter when lipid stores are likely to be more evenly distributed amongst colony members. The figure shows that using this representation the brood ants reach the highest proportion of their capacity in three of the colonies (and are close to full in colony V in-fact) while in colony IV the arena ants reach the highest proportion. Colony IV appears to fill the lowest proportion of its potential capacity. This may be because the hunger level in this colony is lower, perhaps many workers already have food stored, or because the colony is more constrained in how much food it can provide and distribute in 30 minutes compared to the other three colonies. This constraint may arise from having such a large brood pile and not many workers available to forage. A study using labeled food in the wood ant *Formica fusca* L. also showed that the colonies did not reach their potential capacity [78]. Perhaps this was because these ants also had food stored in their crops from before the experiment.

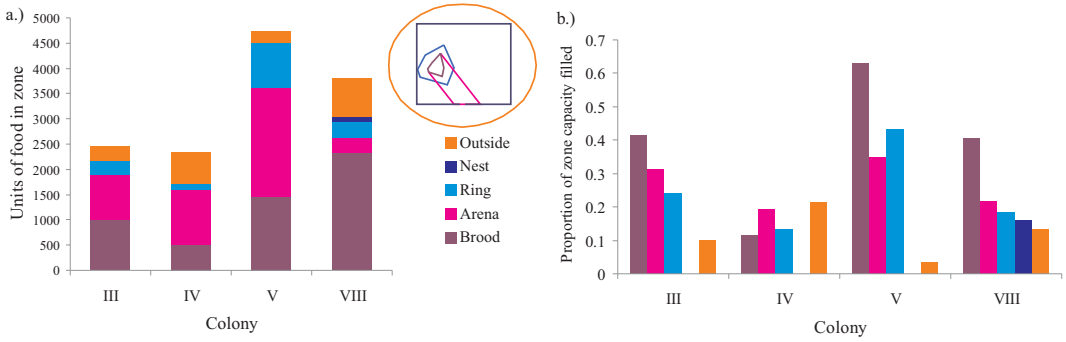

Figure 6-10: *a.) Amount of new food provided to each spatial zone, b.) proportion of the capacity of each zone reached during the famine relief treatment.*

6.3 Transmission pathways

As well as the amounts of food each individual received, in this chapter I will explore the pathways used to transmit the food from the foragers to the workers and the characteristics of these pathways. The existence of chains of transmission during food distribution was proposed by Wilson and Eisner [73]. Figures 6-11, 6-12, 6-13 and 6-14 show the cumulated transmission networks for all four colonies under control and under the famine relief treatment split into background food and new food. It is immediately apparent that there is an enormous amount of feeding occurring under famine relief compared with control. What is also clear is that transmission under famine relief does not appear strongly partitioned or ordered as in figure 6-1 as might be expected. Instead, the famine relief new food network for each colony seems an incredibly tangled web of interactions forming a giant connected cluster which potentially leaves the colony vulnerable to harmful substances. In comparison, the control networks are mostly comprised of smaller disconnected components. The unfed ants in the famine relief networks for the new food are internal ants in three of the colonies. In colony V the three unfed ants are all external ants and may have foraged for food themselves.

It is apparent from the transmission networks for new food under the famine relief treatment that there are some very active foragers in particular in colony IV, ants 83 and 85 appear to be undertaking a large number of the donations. Interestingly in colony V there doesn't appear to be such a dominant forager highlighting a difference in famine relief strategy between these two larger colonies. This aspect is highlighted by looking at the out-degree distributions of the new food networks, seen in figure 6-15, which shows a much larger maximum out-degree in colony IV compared with V while colonies III and VIII are similar (when colony size is taken into account). It is interesting to note that the most active foragers during the famine relief treatment are not necessarily active during the control treatment. For example ant 85 in colony IV does not feature in the control treatment network but is one of the main foragers during the famine relief treatment, see also for example ant 48 in colony III and ant 54 in colony VIII.

The in-degree distributions, figure 6-16, show that colony V has a high proportion of ants with zero in-degree. This is because this column includes foragers which did not receive food and there is a high proportion of such foragers in colony V. The in-degree gives an idea of an individuals direct exposure to other ants and

shows that colonies III and VIII peak at an in-degree of 3 and 2 respectively while IV and V have much flatter distributions ranging up to a maximum of 10. Exposing an individual to ten other ants may be a risky strategy but this does not differentiate between direct exposure to foragers (which may be more dangerous) and direct exposure to non-foragers (which may be less dangerous). Exposure to foragers is looked at in more detail in section 6.4.

The positioning of the nodes in figures 6-11, 6-12, 6-13 and 6-14 is achieved using a technique called "spring-embedding" which places nodes using an iterative algorithm treating each node as a weight on a spring [116]. This technique usually produces graphs which are easier to read than a random positioning technique (I have also moved a few nodes by hand to make them easier to see!).

6.4 Protection during food transmission

Chapter 1 outlines how social insect colonies are particularly vulnerable to exploitation by pathogens. The main reasons for this are that they live at high densities over long periods of time and the members are often genetically very similar. In addition to this, during the famine relief treatment a large amount of food is introduced to the colony very quickly through many interactions between individuals creating a potential opportunity for a pathogen or parasite to exploit. It is therefore relevant to ask whether the network structure used during the famine relief process is somehow adapted to provide security against a harmful substance. I will now explore the transmission process and networks in the context of how exposed to risk the colonies are during the distribution of new food and how they might minimize the risk posed by such rapid transmission.

On first inspection the transmission networks appear not to be structured for risk management as the majority of the colony members are in the single connected component. However, it is important to note that these representations contain all the interactions within the 30 minutes of the famine relief treatment and therefore not all the pathways represented are possible. As an example, in figure 6-17 it appears that individual C is exposed to both individuals A and B. However, if B fed individual C before A fed B then the pathway from A to C through individual B is not causally possible in this instance. The analysis in this section takes into account causality in the transmission pathways.

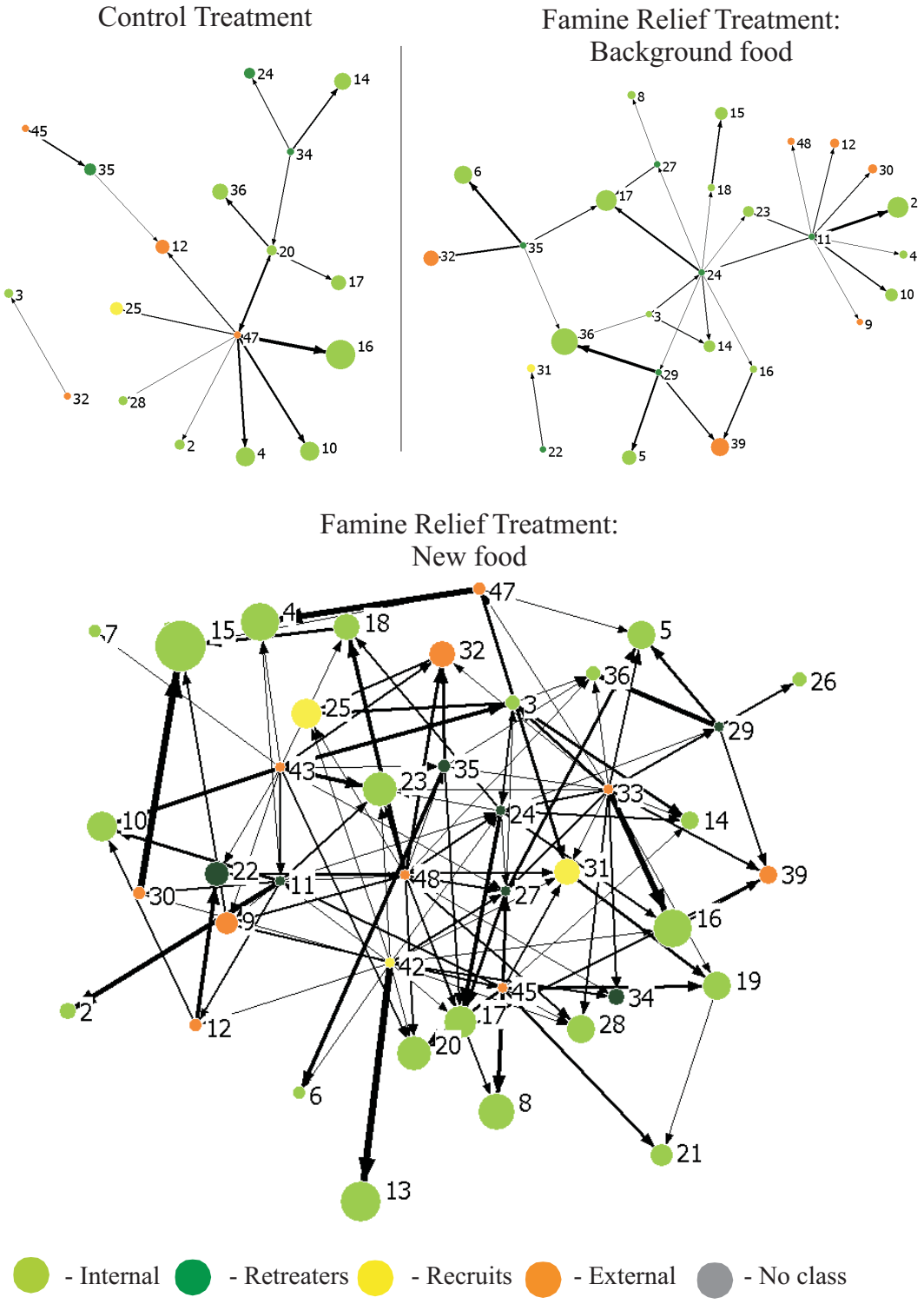

Figure 6-11: *Food distribution networks for colony III, N_C=42. Size of nodes indicate the net-food the individual had at the end of the treatment. Width of arrows indicate tie-strength. Colour of nodes based on spatial classification, see figure 5-12.*

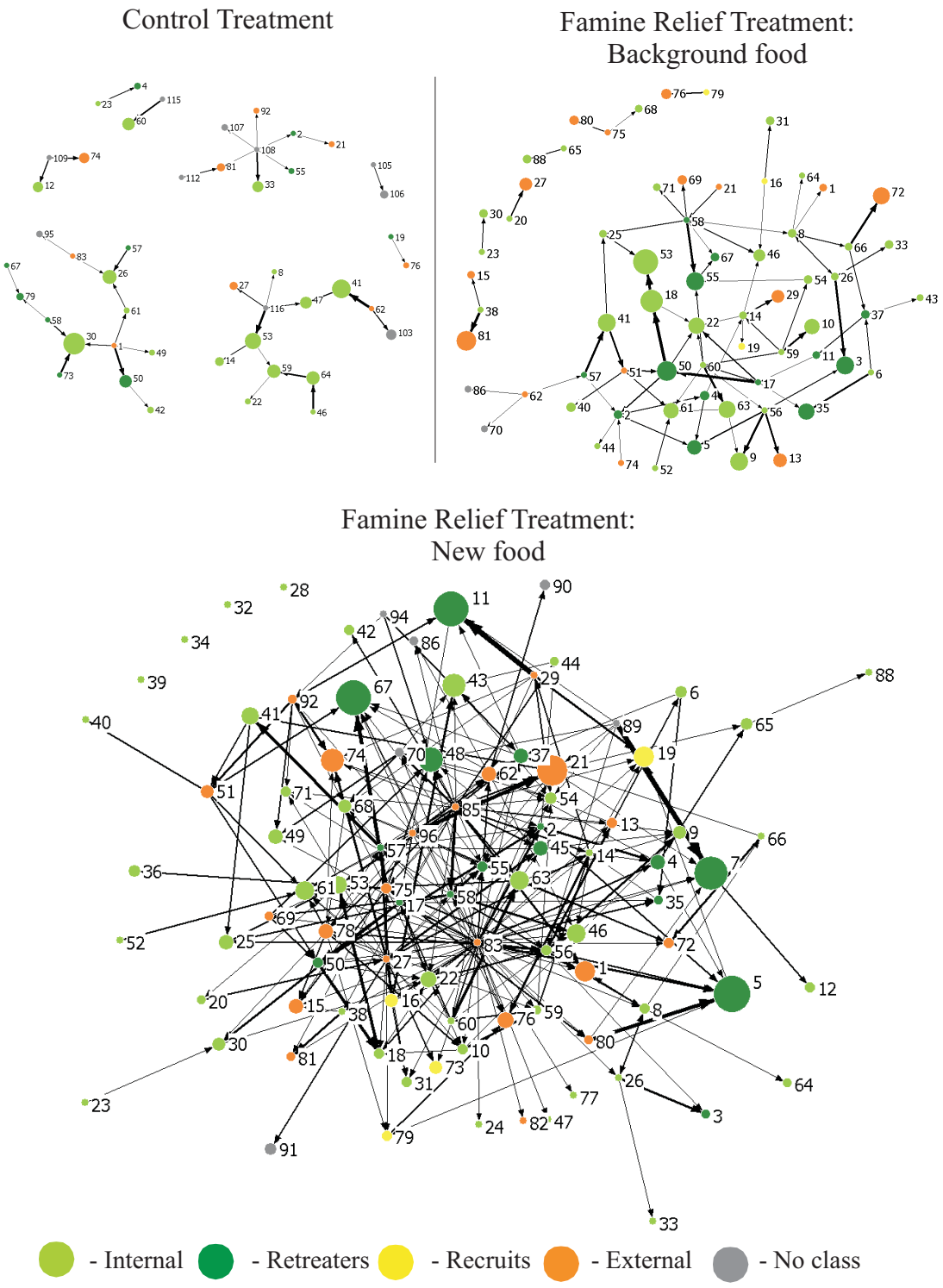

Figure 6-12: *Food distribution networks for colony IV, N_C=95. Size of nodes indicate the net-food the individual had at the end of the treatment. Width of arrows indicate tie-strength. Colour of nodes based on spatial classification, see figure 5-12.*

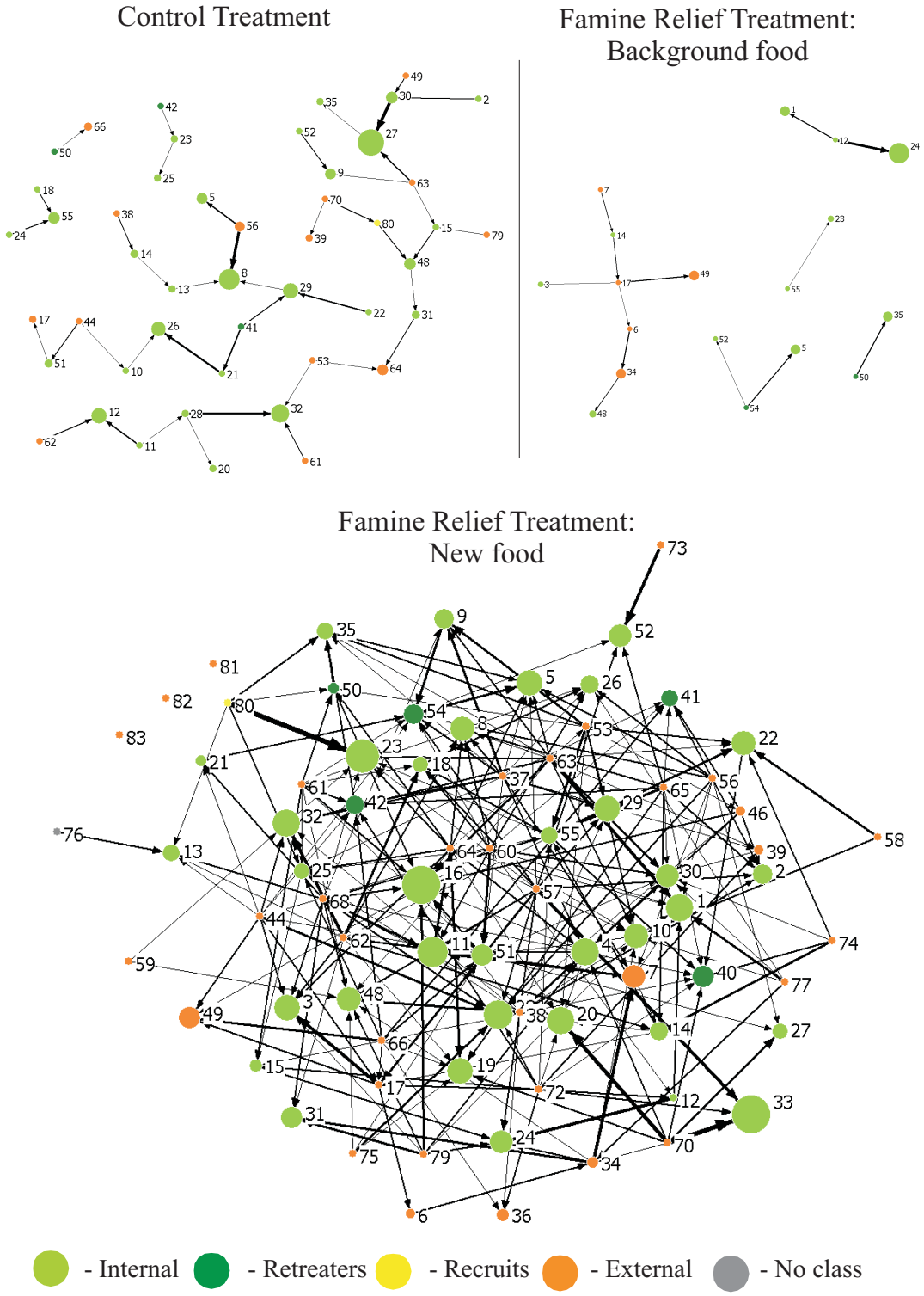

Figure 6-13: *Food distribution networks for colony V, N_C=77. Size of nodes indicate the net-food the individual had at the end of the treatment. Width of arrows indicate tie-strength. Colour of nodes based on spatial classification, see figure 5-12.*

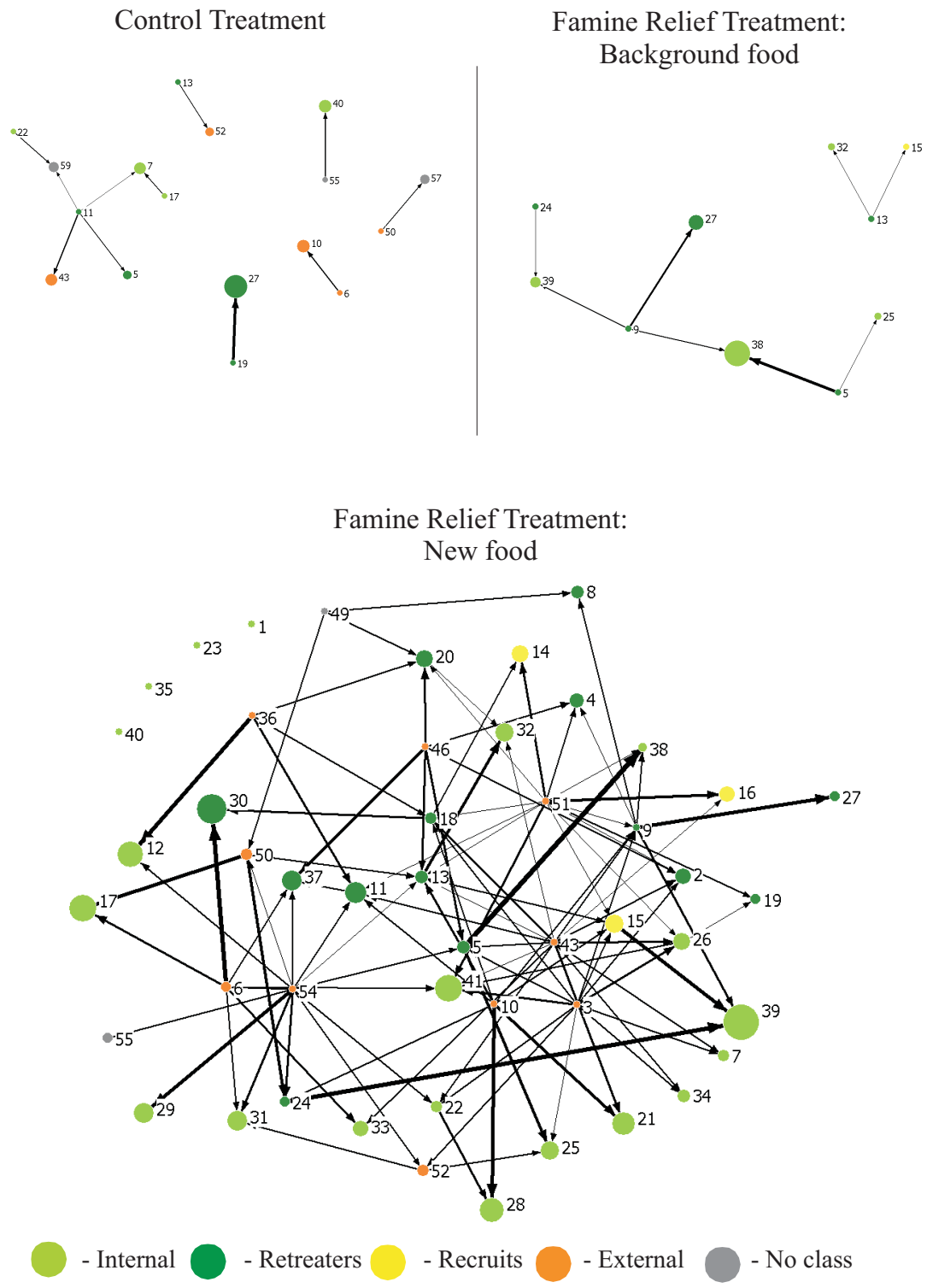

Figure 6-14: *Food distribution networks for colony VIII, N_C=49. Size of nodes indicate the net-food the individual had at the end of the treatment. Width of arrows indicate tie-strength. Colour of nodes based on spatial classification, see figure 5-12.*

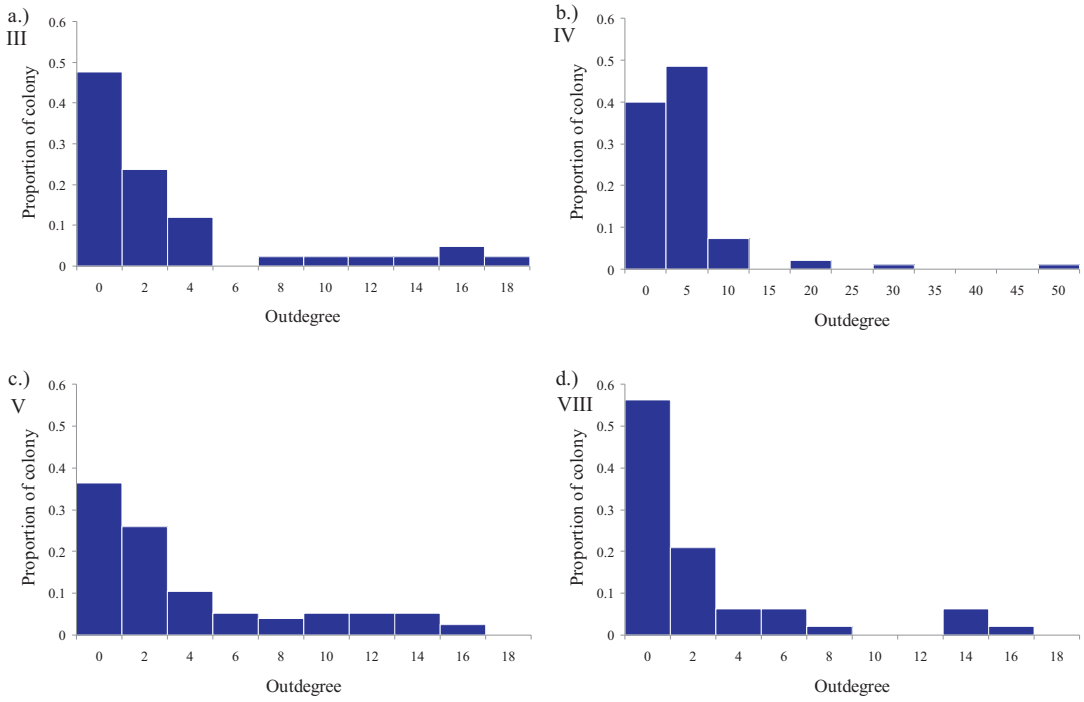

Figure 6-15: *Out-degree distribution of ants for the transmission of new food during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

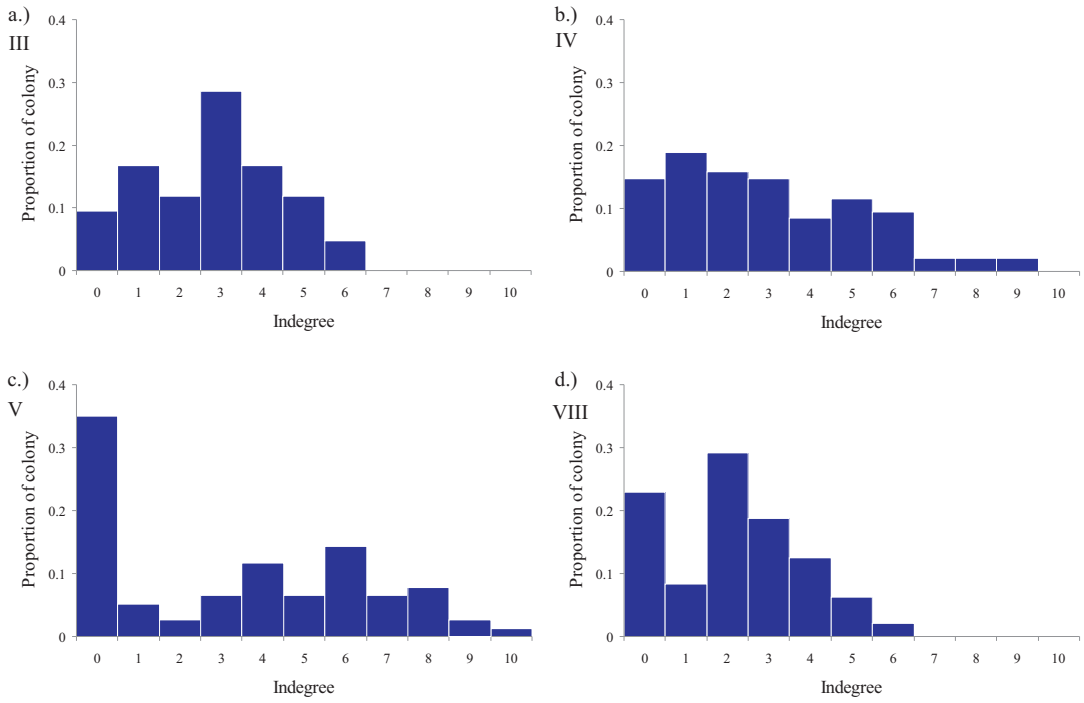

Figure 6-16: *In-degree distribution of ants for the transmission of new food during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

Figure 6-17: *Diagram to demonstrate causality: Individual C is only exposed to A if the feeding event between A and B occurs before that between B and C.*

Exposure in the transmission networks can be approached from two directions, firstly, from a 'top-down' approach for example taking a 'source' (a forager that provides new food during the famine relief treatment) and looking at how much of the colony they reach directly and indirectly, see figure 6-18 a.). Secondly by a 'bottom-up' approach, for example by taking a non-source and seeing how many sources they are exposed to directly and indirectly, see figure 6-18 b.).

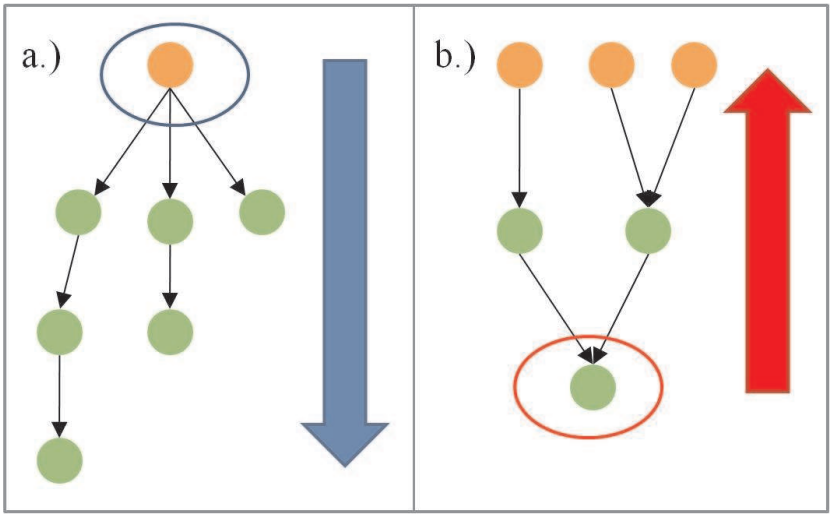

Figure 6-18: *Diagram showing a.) top-down and b.) bottom-up approaches to looking at the transmission networks. The source in a.) reaches 6 individuals: 3 directly and 3 indirectly. This is known as the 'out-domain' of a source and its size is number of individuals reached, i.e. 6 in this case. The non-source in b.) is exposed to 3 sources indirectly.*

I will first use a 'top-down' approach and explore the reach of the sources. It is useful to know how many sources there are in each colony and the amount of new food they are each responsible for providing. Figure 6-19 shows the distributions of the amounts of new food the sources provided for each colony. It shows that in colony IV there are two main sources providing a large amount of food (around 500 units), in contrast in colony V there are lots of sources but all provide only a relatively small amount (up to 350 units). In colony III there is a smaller number

of sources, one providing 500 units and several providing between 200 and 400 units. In colony VIII there is also a fairly small number of sources but several are providing a large amount of 500+ units. This figure also highlights that only a small number of the sources in each colony are 'recruits' (ants that were internal during control and external during the famine relief treatment). This suggests that recruiting a large number of new foragers from inside the nest is not the main strategy employed for relieving the famine. Recruiting a large number of nest-mates may not be necessary in this scenario as the food source is only a short distance from the nest (1cm). This distance could be varied in future experiments to see whether more nest-mates are recruited if the new food source is placed a long distance from the nest.

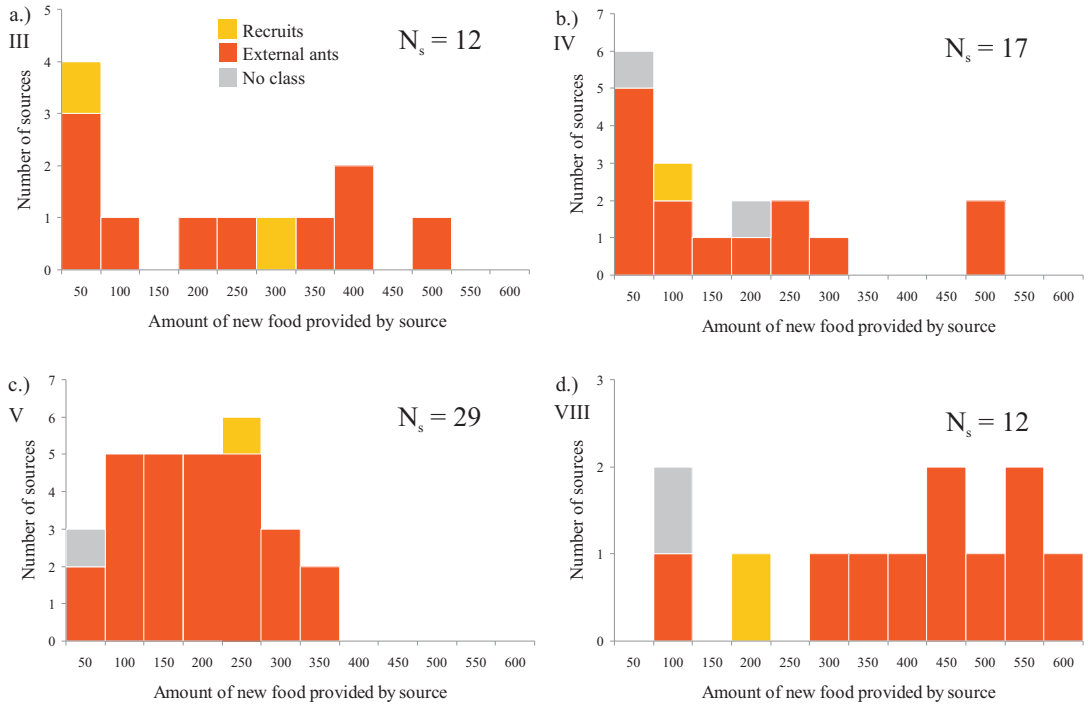

Figure 6-19: *The amount of new food provided per source during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

In theory, one of these sources might reach a high proportion of the colony when indirect exposure is taken into account. However, the amount of food transmitted might play an important role here. A small amount of food may not be a dangerous amount if there is a dilution effect. Figure 6-20 shows the distribution of amounts of new food transferred per event in each colony. It shows that most events in colonies III, IV and V transmit around 20 units or less to the recipient with a small number greater. In colony VIII around 70% of the events

transmit less than 40 units while the rest are larger amounts ranging up to 180 units. This is curious in itself and could be a risky strategy for colony VIII if the foragers cannot determine whether the food they have collected contains harmful substances such as pathogens or poisons, see [55].

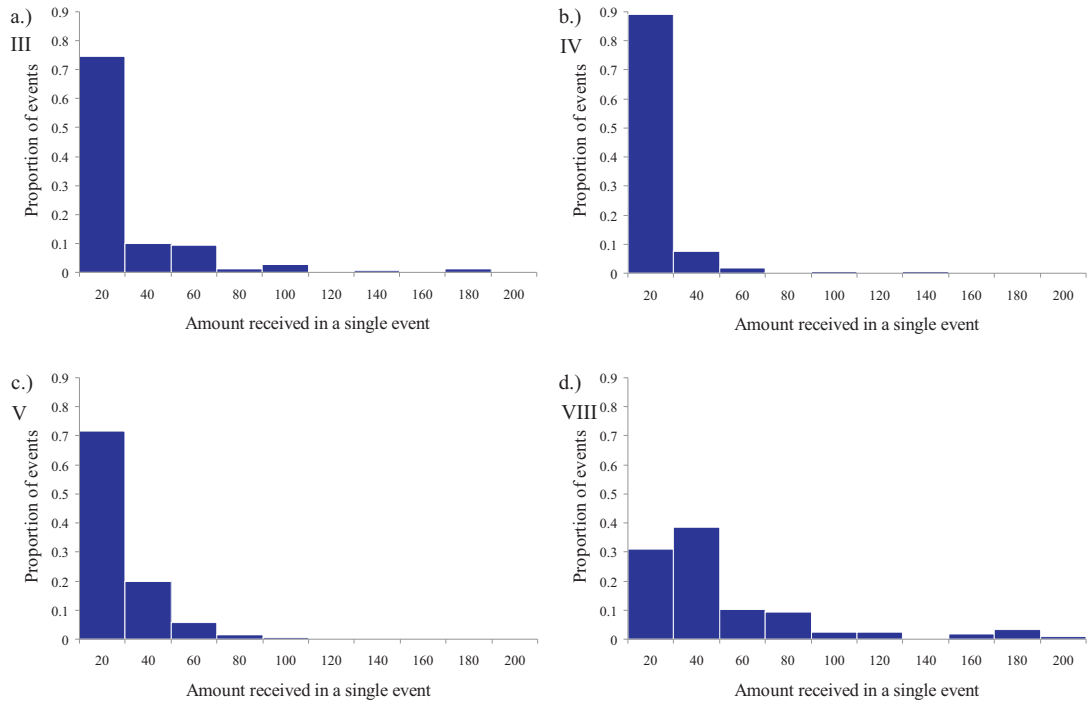

Figure 6-20: *The amount of food (new food) received per event during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

Using the information gained from looking at the amounts transmitted per event, figure 6-20, the exposure matrix, $\mathbf{E}[N_c, N_c, t]$, was filtered to remove events less than a threshold amount ranging from 0 units (i.e. keep all events) up to 20 units. The reach of the sources at these different filter thresholds is shown in figure 6-21 and shows that filtering causes dramatic reduction in the proportion of the colony that the biggest source can reach. In colonies III and IV removing events that were less than 5 units reduces the reach of the biggest source from over 65% of the colony down to 45% of the colony. Colony VIII does not show such dramatic reductions because the feeding events in this colony tend to transmit larger quantities of food as shown in figure 6-20. Colony V also does not show such a dramatic reduction in reach however the maximum reach of a source in this colony is relatively low, around 35%, compared with the other colonies. Figure 6-22 shows, as an example, how the out-domain of the largest source in colony III decreases as the filter threshold is increased.

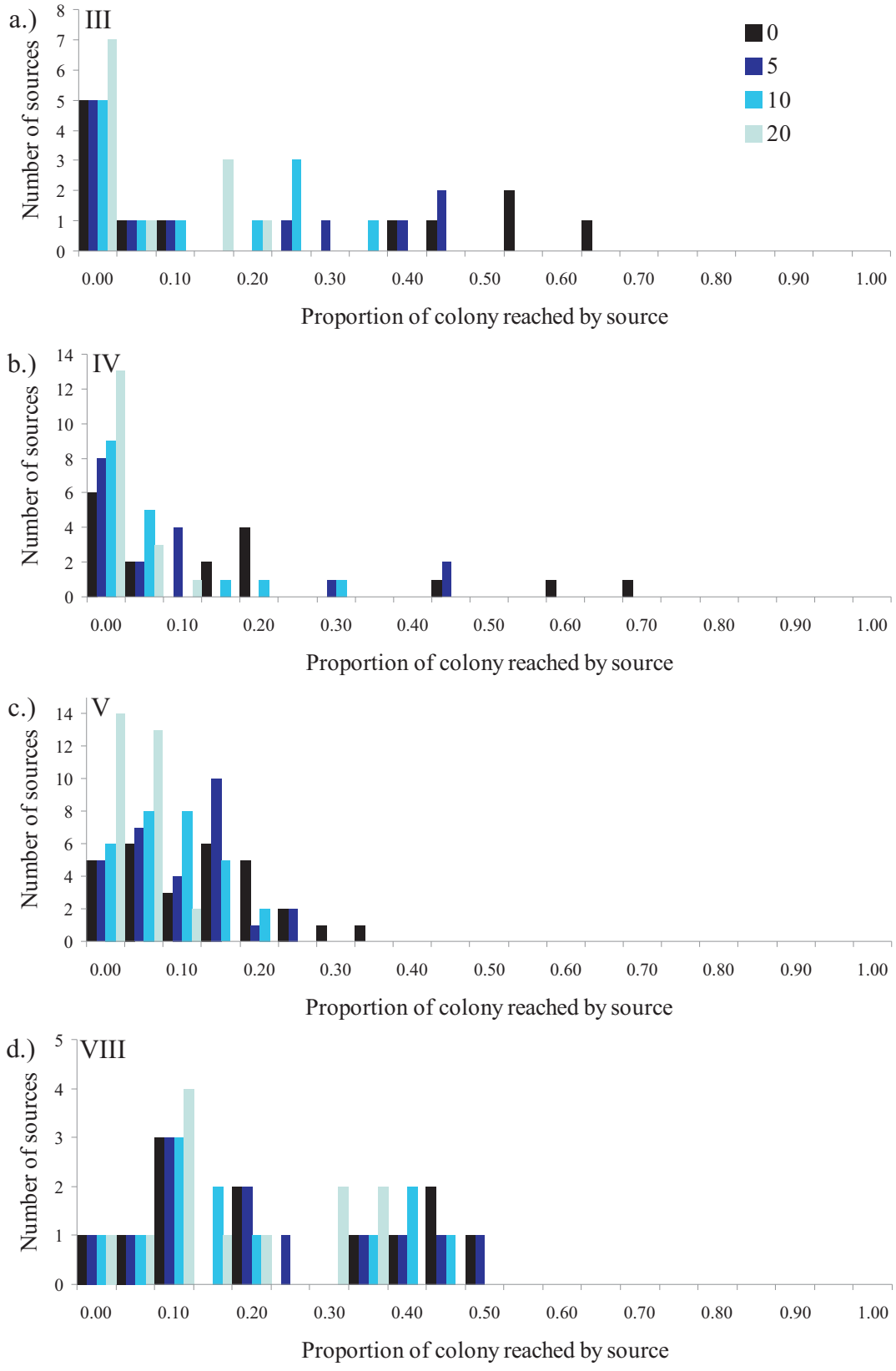

Figure 6-21: *The proportion of the colony that sources reach during the famine relief treatment at different filter thresholds, 0 to 20 units of new food. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

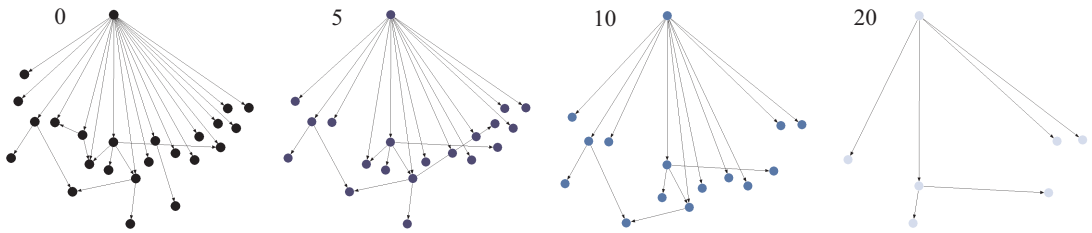

Figure 6-22: *An example of the out-domain of a source as filter threshold increases from 0 to 20 units. This is the out-domain for ant 33 in colony III.*

The reach of sources is a 'top-down' measure for looking at the exposure in a colony, I will now present a 'bottom-up' approach by looking at how many sources individuals are exposed to. Figure 6-23 shows the number of sources ants are exposed to during the famine relief treatment. As in figure 6-10 the ants are separated into classes based on the spatial zone they used the most during the control treatment. If we ignore the classes initially and look at the shape of the distributions, of the ants that are exposed to sources in colony III and IV the modal class is exposure to 3 sources, in colony VIII it is to 4 sources and in V it is to 6 sources. This higher number in V is likely to be because there are a higher number of sources in this colony, however we saw from figure 6-21 that each source only reaches at most 35% of the individuals in colony V. All external ants during the famine relief treatment at least have the potential to be sources themselves, so if we ignore the orange bars in figure 6-23 we see that of the internal ants in colony VIII the modal class shifts from 4 to 2. We might expect the brood ants (purple bars) to be exposed to fewer sources than other ants as these ants are in direct contact with the brood pile. However, we see from the figure that some brood ants are exposed to a large number of sources in all four colonies. What this representation does not show is whether these individuals are directly exposed to this number of sources. Direct exposure may be more risky than indirect exposure if the intermediate ants in the transmission chain act as testers for the food and if direct exposure to a forager increases the risk of contracting an external parasite. The pathlength of an individual from a source in the transmission chain tells us whether they are directly or indirectly exposed to a source.

Figure 6-24 shows for each colony of the average reachable pathlength of individuals from a source in the transmission network during the famine relief treatment separated again by spatial class under the control treatment. The figure shows that in colonies III, IV and V the brood ants are further away from the sources than the arena and ring ants but this pattern is not true for colony VIII. This is

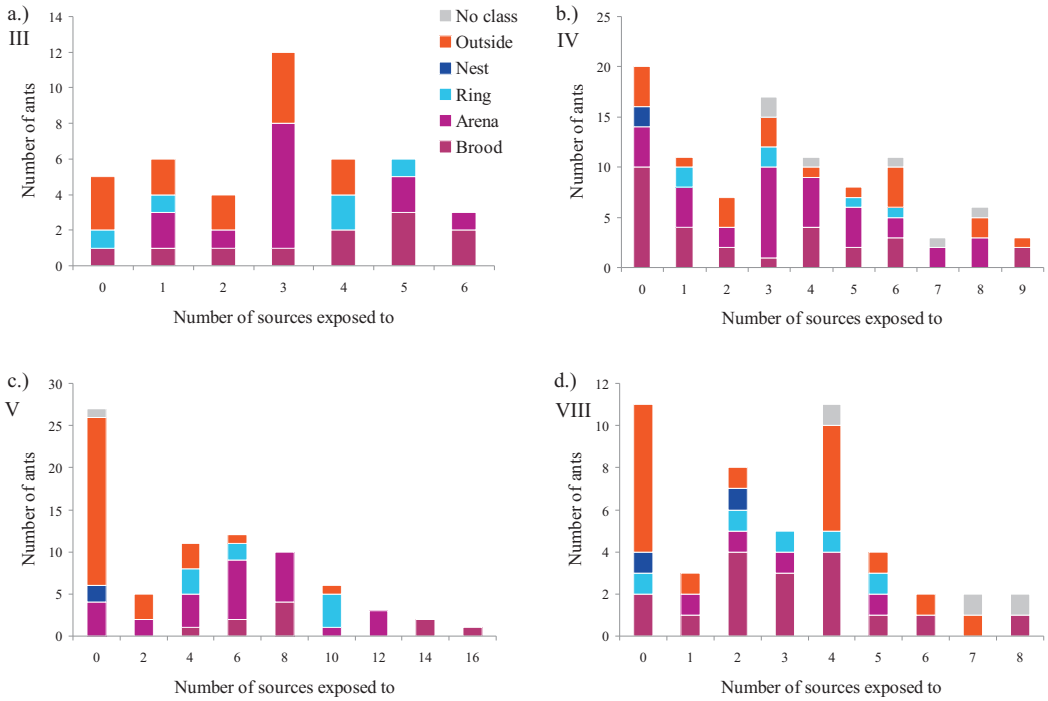

Figure 6-23: *The number of sources ants of different spatial classes are exposed to during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

potentially a risk management strategy by colonies III, IV and V by feeding the brood ants via intermediate workers instead of directly by the foragers. However, it could also be the case that it is simply more efficient for these brood ants to remain close to the brood and wait for an intermediate ant (not a forager) to come to them with food. This results in the brood ants being further down the chain of transmission due to the spatial organisation. We know that not all the brood ants do this as we have seen in Chapter 3 that the ants abandon the brood during the frenzy period in the famine relief treatment and some are likely to receive directly from foragers at this point. This explains the variation in path-length for the brood ants in colonies IV, V and VIII seen in figure 6-24.

Combining these two pieces of information figure 6-25 shows the number of sources individuals are exposed to against the average path-length from a source. The number of sources an individual is exposed to increases with the average path-length from source, see table 6.3. In other words, ants which are exposed to many sources tend not to be directly exposed to the sources, they are further down the chain of transmission. (Although note the low R^2 values as there are several ants that are exposed to many sources at an average path-length of 1.)

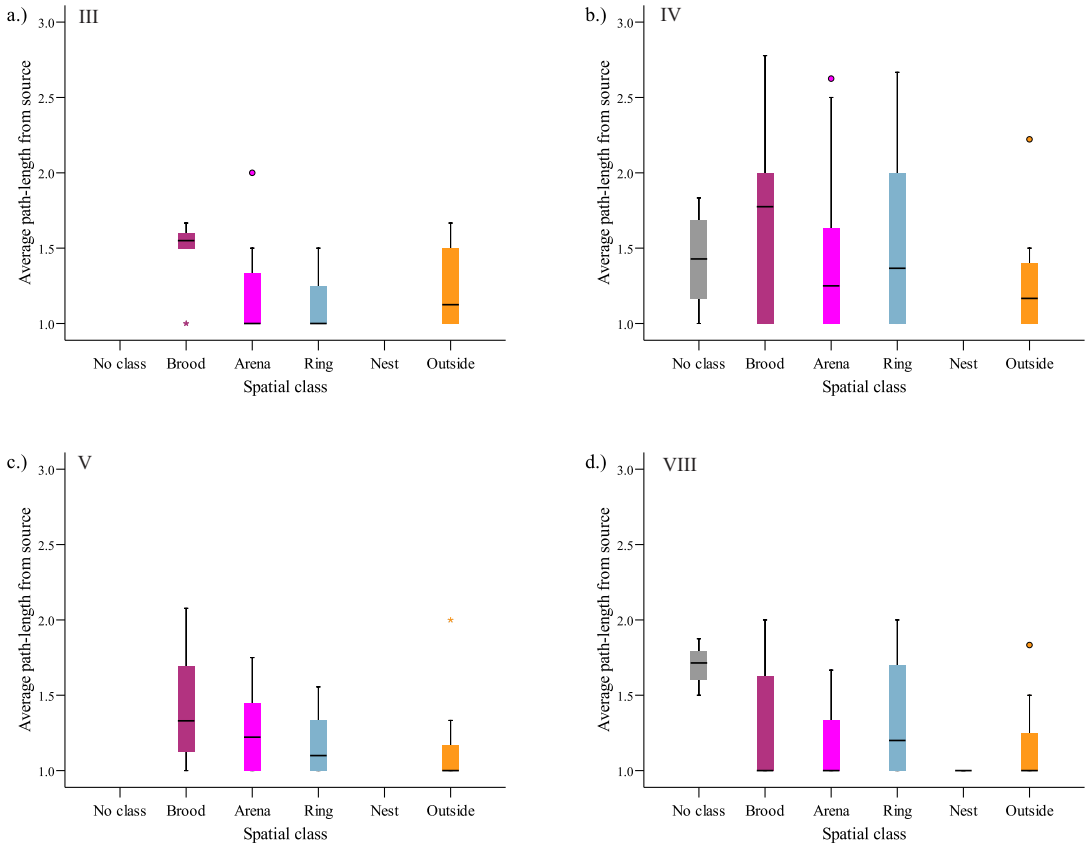

Figure 6-24: *The average path-length from reachable sources during the famine relief treatment. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

There does not appear to be a clear pattern for spatial class with respect to pathlength from source and number of sources exposed to. Perhaps mixing the levels and types of exposure to sources promotes robustness within the different spatial zones.

6.4.1 Overlap of sources

One of the ways in which the ants might reduce the risk of spreading a pathogen during the famine relief process is to partition the transmission network of incoming food, see figure 6-1. In this way ants are only exposed to the forager in the partition to which they belong. On initial inspection the transmission networks for new food, shown in figures 6-11, 6-12, 6-13 and 6-14, do not appear as though they could possibly be partitioned. However, it is important to remember that these representations are accumulated over all the interactions during the 30 minutes and it is impossible to pick out the causal pathways from these alone.

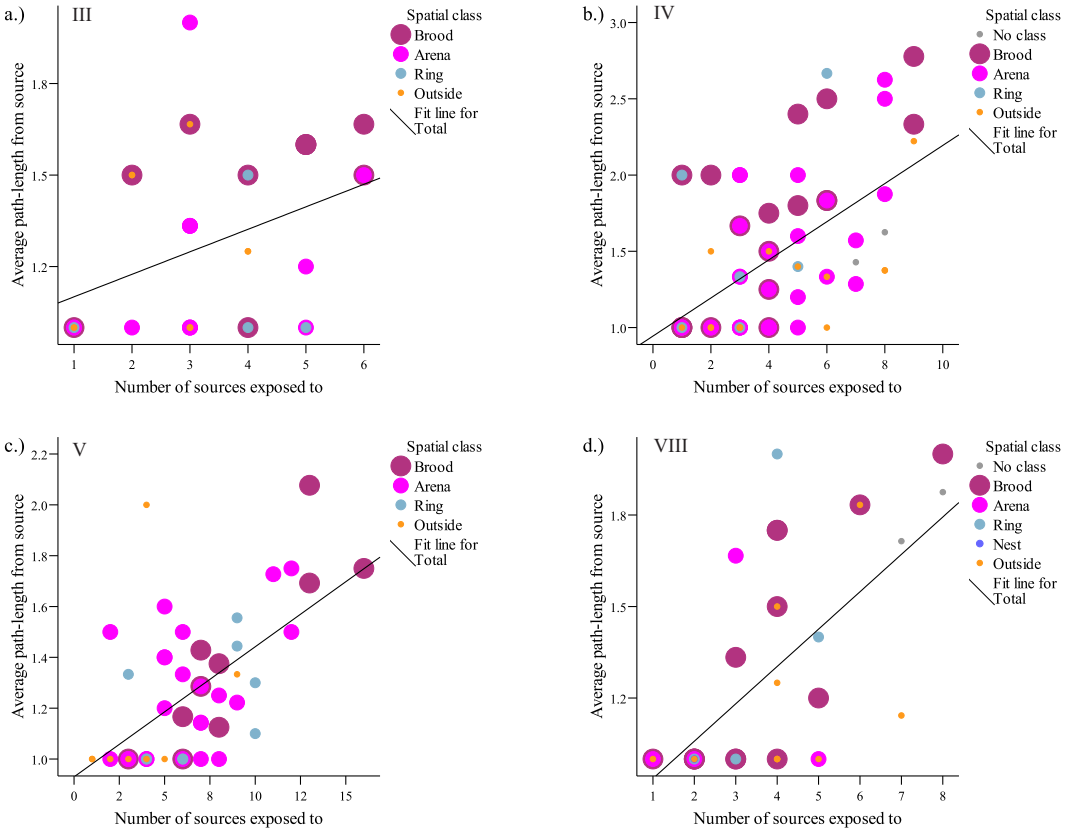

Figure 6-25: *Number of sources ants of different spatial classes are exposed to during the famine relief treatment against the average path-length from reachable sources. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

A way to look at the level of partitioning present in the transmission network is to consider the overlap between the out-domains of the sources. The size of the out-domains was looked at in figure 6-21. An overlap between two sources, A and B, is created when individuals in the out-domain of source A are also exposed to source B. For example, the ants in the dashed oval in figure 6-26 are in the overlap between the sources A and B.

A measure of how much overlap, ω, there is between sources can be calculated using the out-domains of each source. If the colony is entirely partitioned then the sum of the sizes of the out-domains of all sources will be equal to the number of fed ants, i.e. $\sum_{i=1}^{N_s} d_i = N$, where N_s is the number of sources, d_i is the size of the out-domain of each source and N is the total number of fed ants. So a measure of the overlap, ω, could be the sum of the out-domains minus the total number of fed ants, $\omega = \sum_{i=1}^{N_s} d_i - N$, which would equal zero if the network was completely partitioned.

Colony	R^2	F	Sig.	Constant	Gradient
III	0.183	8.270	0.007*	1.007	0.078
IV	0.340	38.655	<0.005*	0.941	0.126
V	0.374	29.906	<0.005*	0.933	0.051
VIII	0.411	24.391	<0.005*	0.814	0.122

Table 6.3: *Linear regression analysis on average pathlength from sources against number of sources exposed to, with number of sources exposed to as the independent variable. Asterisks indicate significant results.*

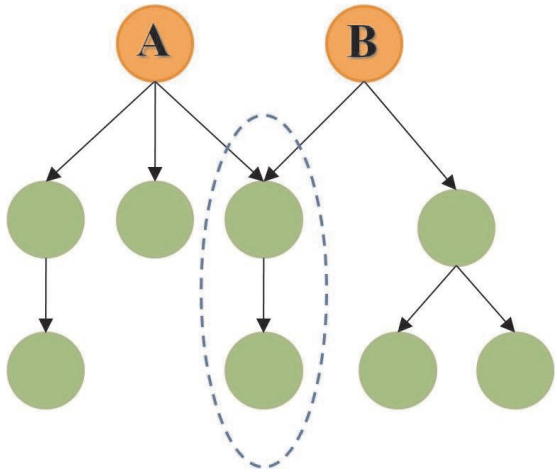

Figure 6-26: *Diagram to demonstrate the overlap between two sources, A and B. The individuals in the dashed oval are in the overlap between the two sources. The remaining individuals are either in the out-domain of source A only, or source B only.*

If the colony is not partitioned and there is lots of overlap between the out-domains of sources the sum of the sizes of the out-domains will be much greater than the total number of fed ants, i.e. $\sum_{i=1}^{N_s} d_i \gg N$. If the out-domains are entirely overlapped, i.e. if all the fed ants appear in the out-domain of every source then the sum of the out-domains will be equal to the number of fed ants multiplied by the number of sources, $\sum_{i=1}^{N_s} d_i = N \times N_s$. Using this logic, ω is normalised by dividing by $(N \times N_s) - N$,

$$\omega = \frac{\sum_{i=1}^{N_s} d_i - N}{N(N_s - 1)}, \qquad (0 \leq \omega \leq 1). \tag{6.4}$$

This normalisation means that when there is no partitioning, i.e. there is complete overlap between sources, ω equals 1, and when there is complete partitioning, i.e. no overlap, ω equals zero. ω was calculated for each of the four colonies

under the famine relief treatment, the results are shown in figure 6-27. Interestingly all four colonies showed an ω value of between 0.19 and 0.26. This is perhaps surprising given the differences in number of sources and reach of sources seen previously.

The overlap measure has not been calculated for the control treatment for two main reasons. Firstly, because the majority of ants are only fed at most once during control the transmission networks are almost entirely partitioned. To get a fair comparison between the treatments of how overlapped the transmission network is would require the same number of interactions or the same number of fed ants. This would require recording feeding interactions during the control for much longer than the 30 minutes used in this study. Secondly, because the food was available outside the nest before the start of the control treatment identifying individuals which drank at the honey solution and then returned to the nest before the start of the video is impossible therefore we cannot calculate the overlap of sources.

6.4.2 Randomisation test for overlap, ω

The values obtained for ω suggest that there is actually little overlap between sources and therefore the transmission networks under famine relief are partitioned to some degree. A test is needed to verify that this is true compared with the level of overlap that would arise if the feeding interactions were allocated to individuals at random. I designed a randomisation test based on the properties of the feeding data from the real experiment to explore this.

In this randomisation the feeding events occurred at the same times with the same amounts of food transmitted as the feeding events in real data for each colony. This meant that the total number of feeding events and the total amount of food transmitted were equal to those of the real data. The randomisation goes through the feeding events from the real data and uses the following rules to select a donor and a recipient for each event:

1. When the donor in the real data was a source the same individual was selected to be the donor in the randomisation.

2. When the donor was a non-source the randomisation selects a non-source out of those individuals who have previously received enough food during that run of the randomisation to donate the amount donated in that feeding event. In addition, the number (but not the identity) of non-source donors was limited to that in the real data so that if the number of non-source donors was reached and there were none available to donate then the run was deemed impossible (Number of non-source donors in each colony in the real data: III = 10, IV = 29, V = 19, VIII =9). If there were no available non-sources with enough food then the run is deemed impossible and a new run begins.

3. If the recipient in the real data was a non-source then a non-source recipient is selected from the ants that were fed in the real data.

4. In the real data, there were a few ants in each colony that never received food, these were never chosen to be recipients in the randomisation so that the maximum value for N that can be achieved in the randomisation is the number of fed ants in the real data.

5. If the recipient was a source in the real data a source is selected from those that received in the real data (Number of sources which receive food in the real data: III = 8, IV = 10, V = 6, VIII = 4).

6. For all cases individuals could only be selected if they were not currently involved in a feeding event. If no appropriate individuals were available for a feeding event (donors or recipients) the run was deemed impossible, not counted and re-started.

1000 runs were completed for each colony. Figure 6-27 shows that compared to the randomisation data the combined real data are significantly less overlapped. This indicates that in the real colonies there is weak partitioning occurring. Figure 6-28 shows the distributions of values of ω obtained from the randomisation. While ω has been devised so that it is possible to range from 0 (completely partitioned) up to 1 (completely overlapped), these distributions show the possible range of values of ω within the constraints of the randomisation is much narrower.

While ω is a useful overall measure of the amount of overlap between all the sources in a colony, it is also interesting to look at the pairwise overlap between sources given that there is a range in out-domain size, seen from figure 6-21. It

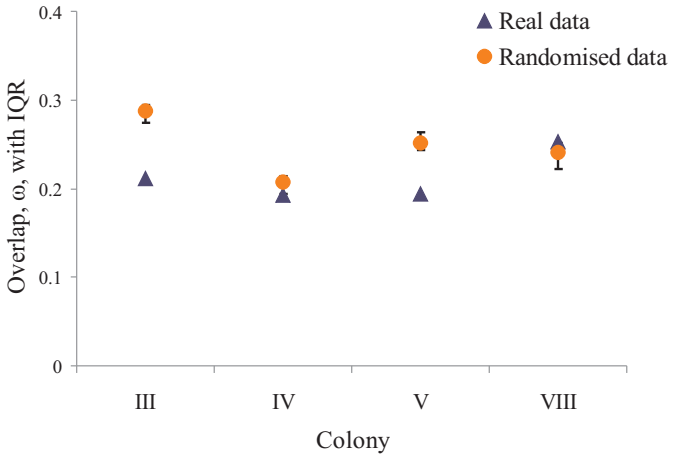

Figure 6-27: *Overlap measure from real data compared with the median value and inter-quartile range from the randomisation for each colony. Fisher's combined method* $\chi^2_8 = 32.439, p < 0.01$.

is possible that the smaller sources are contributing disproportionately to ω and lowering it. Figure 6-29 gives the distribution of pairwise overlap between sources in each colony. The pairwise overlap is taken as the proportion of fed ants that are present in the out-domain of both sources. The figure shows that this pairwise overlap varies from zero up to around 0.4 in three of the colonies, III, V and VIII. In colony IV the pairwise overlap ranges up to nearly 80% of the fed ants in the colony. This large overlap would be between the two largest sources in this colony, ants 83 and 85, which feed a large proportion of the colony. Meanwhile, many of the pairwise overlap values are zero, these are potentially between pairs of smaller sources. What this analysis shows is that while ω, the overall overlap across all sources, may be low, between the sources that reach large proportions of the colony the pairwise overlap is high. This is potentially still risky if sources were feeding at different sites and could pick up harmful substances. Perhaps the colonies are trying to facilitate the mixing of exposure to different sources to facilitate the spread of social immunity, or perhaps the colony is able to detect that all the food brought in by the foragers is from the same site so there is no need to strictly partition what is brought in.

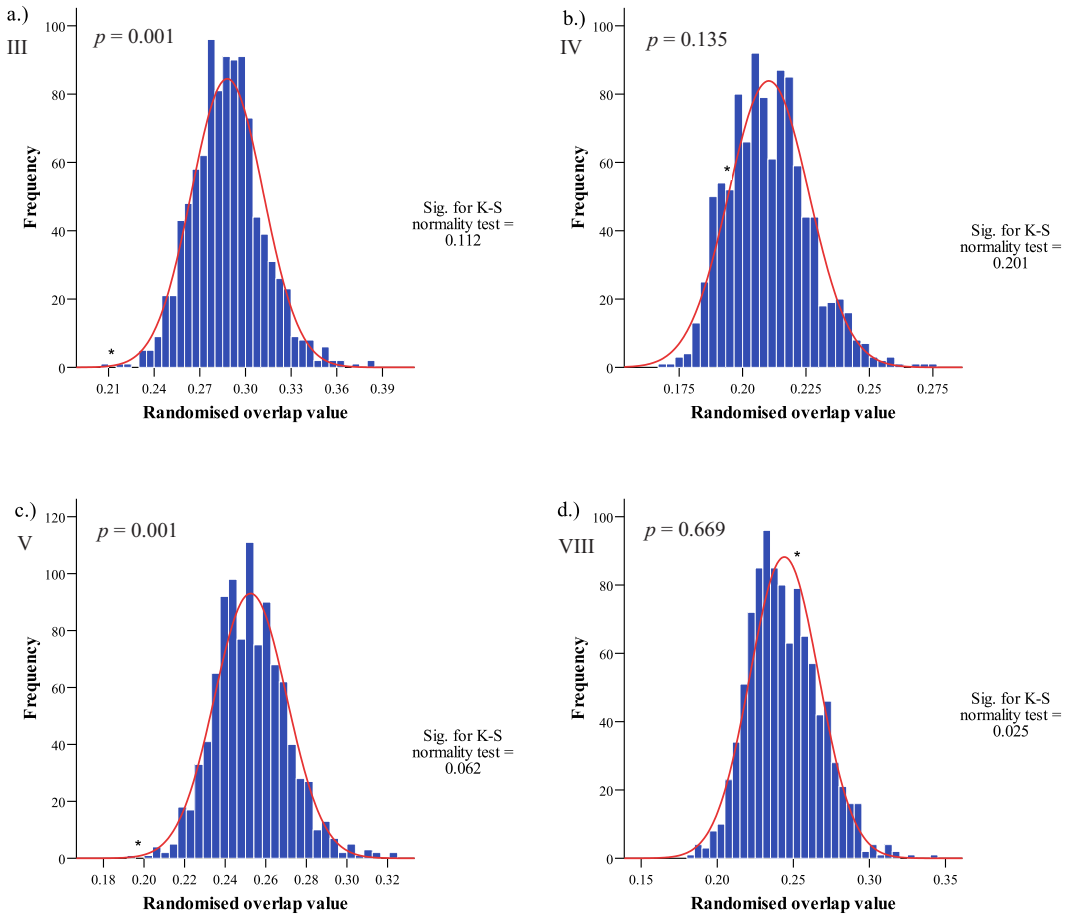

Figure 6-28: *Distributions of overlap measure, ω, for the randomisation data for each colony. Asterisk indicates ω for the real data in each case. Probability values give the number of cases equal to or less than the real data. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49*

6.5 Background feeding and other feeding roles during famine relief

For the final analysis in this chapter I will return to the subject of background feeding to investigate which ants donated a large amount of this background food and the other feeding roles undertaken during the famine relief process.

Figure 6-30 shows the distribution of the amounts of background food that individuals provided. It shows that in colonies III and VIII there are 3 and 2 individuals respectively that provide a large quantity of background food. In comparison in colony IV there are several individuals that provide relatively large amounts of background food for this colony but the amounts are smaller compared to

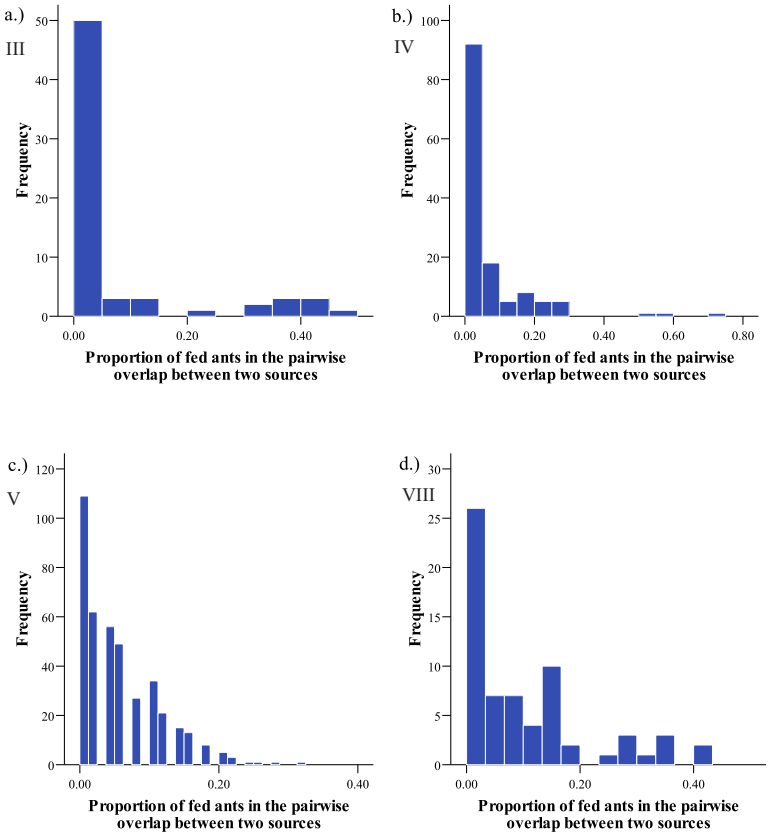

Figure 6-29: *Distributions of pairwise overlap between sources. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

those in III and VIII. In colony V there is only one individual that provides a relatively large (for this colony) amount of food. The colours of the bars indicate the spatial behaviour of the individuals based on whether they were internal or external during the two treatments, see section 5.3 figure 5-12. They show that in colonies III and VIII the large providers of background food are 'retreaters', ants which were external during the control treatment but internal during the famine relief treatment. In IV the large background food providers are a mix between internal ants and retreaters and the one large background food provider in colony V is an internal ant. This indicates that it is not strictly necessary for an ant to have been external during the control treatment to subsequently provide background food during the famine relief process however the individuals providing the largest amount (over 180 units in colonies III and VIII) were external during the control treatment.

Figures 6-31, 6-32, 6-33 and 6-34 show the new food and background food timelines for the largest providers of background food in each colony (with background food becoming negative when the individual provided it). I have taken the largest

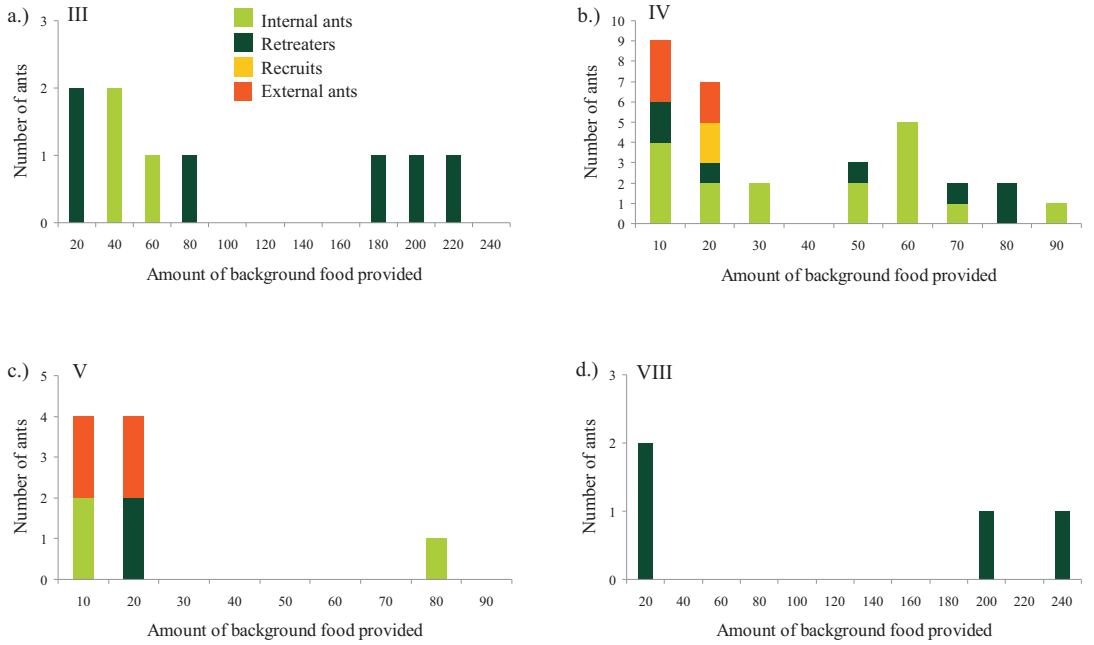

Figure 6-30: *Amount of background food individuals provided during the famine relief period. a.) Colony III, N_c=42; b.) IV, N_c=95; c.) V, N_c=77; d.) VIII, N_c=49.*

providers (80 units or above) in each colony: three in colony III, three in colony IV, one in colony V and two in colony VIII. When we examine more closely what happens to these individuals during the 30 minutes we find that several receive a small amount of new food from a forager before they donate the large amount of background food which they contain. It appears in some cases that these small receptions from an incoming forager trigger the ants to donate their stored old food and in all cases the ants have a relatively small feeding event before they start donating their background food.

There may be several reasons why these ants donate the large amount of stored food they contain. It may be that they pass on the stored food so they can act as foragers themselves and bring in more new food. None of these ants left the nest within the 30 minutes of the famine relief treatment (and the few that I watched beyond the 30 minutes remained inside the nest). This indicates that they may be remaining in the nest to facilitate the management of food distribution within the nest as proposed in [88]. They could potentially be spreading the old relatively safe food they contain to mix with the new food to dilute the effect of any harmful substances that might be in the new food. In addition to this, they may be donating the old food so that they are empty and can receive a large amount from an incoming forager and help distribute the food while the foragers leave

the nest to collect more. It appears as though the ant in figure 6-32 c.) may be undertaking this role shown by the reception of new food at $t \approx 1400$ seconds and subsequent donation at $t \approx 1700$ seconds.

The colonies were not observed during the starvation period, the 48 hours between the control and the famine relief treatments. It is possible that colony V, which does not provide much background food during the famine relief treatment, distributed its stored food during this period and therefore had none to donate during famine relief. This may explain why there was such a large number of sources of new food in this colony relative to the others.

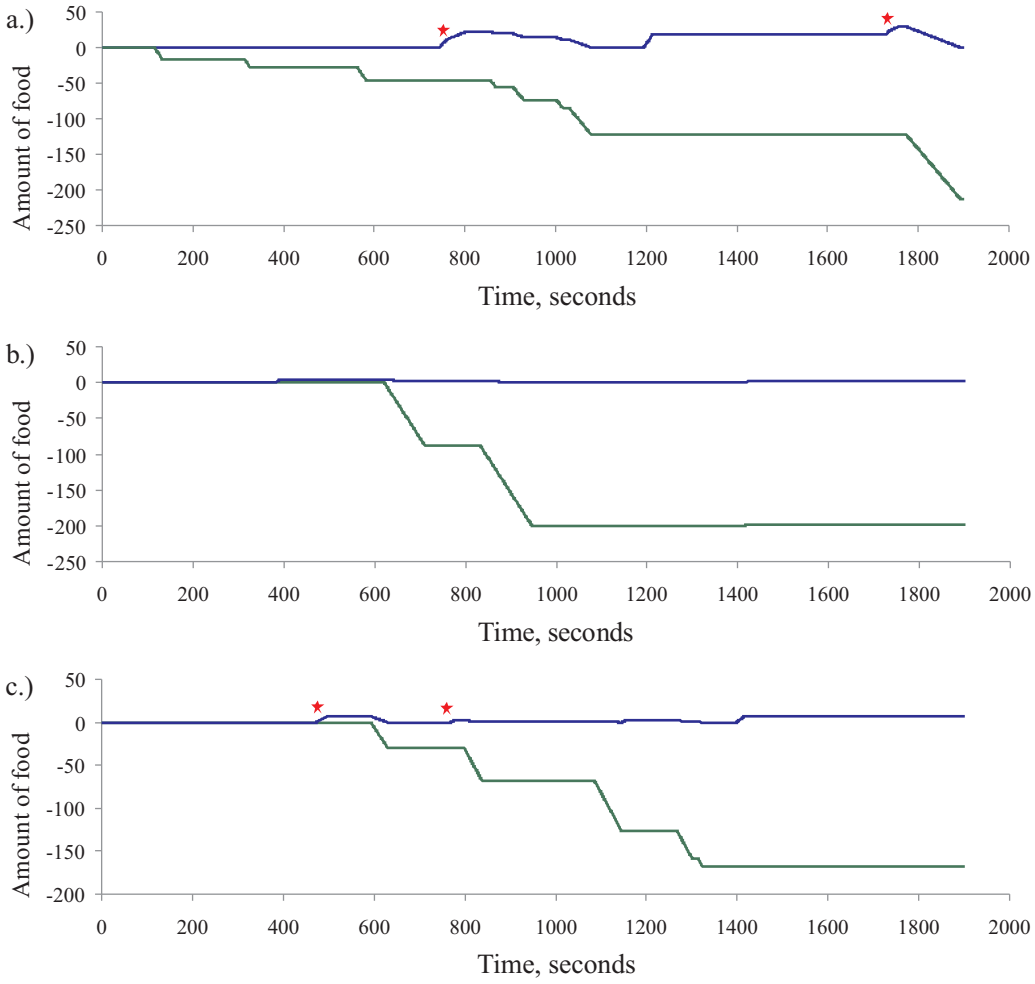

Figure 6-31: *Timelines for the largest providers of background food in colony III, a.) ant 11, b.) ant 29, c.) ant 35. The blue line represents the net amount of new food, while the green line represents the amount of background food (which becomes negative when donated). The red asterisks indicate the small reception from an incoming forager that triggered the ant to donate their stored load.*

6.6 Summary

This chapter has addressed objective C: to determine amounts of food received, whether an even distribution of food among workers is achieved and whether colony capacity is reached; and objective D: to deduce the structure of transmission pathways and whether there is a preference for who feeds whom. Previous studies have suggested that the duration of a trophallaxis event is proportional to the amount of food transferred from the donor to the recipient [117, 37]. This chapter has provided preliminary evidence that this is the case and that the number of recipients receiving simultaneously from a donor does not affect the rate at which she pumps out food, see figure 6-3. Further experimental work is needed to verify this and the exact relationship between duration of trophallaxis and volume of liquid transferred. We have seen that in the famine relief treatment all four colonies provided well over twice the amount of food provided during the same length of time under the control, see section 6.2. However, none of the four colonies filled their potential capacity during the famine relief treatment, see figure 6-10, a result also seen in a study using *Formica fusca* [78]. There may be several reasons that colonies do not fill to their potential capacity with new food, for example, many workers may already have food stored in their crops from before the starvation period (this is difficult to determine without dissection). It may also be advantageous not to fill the entire capacity given that the queen and larvae require different food types to workers [60]. The individuals with the largest net-amount by the end of the famine relief treatment tend to be brood ants, see figure 6-8. This is an interesting result as other studies have found brood workers to receive the least amount of food, e.g. [80]. Perhaps in the current study brood ants required the most food as they had been feeding the larvae during the starvation period and used up their own reserves.

During famine relief most ants in each colony received food in multiple feeding events with similar amounts received regardless of whether it was a first feeding or a subsequent feeding, figure 6-9. Receiving food in multiple events could be a safety mechanism to allow workers to test or dilute the food. Alternatively it may be a way to manage the incoming food so all individuals receive some food quickly before filling up to reach their individual capacity. Another study has shown that brood workers feed larvae in many small increments resulting in a uniform distribution of food across all larvae [64]. Feeding workers in a similar way may eventually achieve a similar result whilst also providing information to foragers about the hunger state of the colony.

This chapter addresses objective D to determine the structure of the transmission pathways. Eusocial insect colonies are particularly vulnerable to pathogens and parasites, see [42, 30, 2, 41]. Studies have shown social interactions, such as allogrooming and trophallaxis, increase the spread of pathogens, [30, 43, 41]. It is therefore probable that trophallaxis provides a route for pathogens to spread during famine relief when there is a high frequency of interactions. Partitioning the transmission network for the new food could be a way to reduce such spread. This was investigated by looking at the overlap in the out-domains of sources and revealed an overall level of overlap lower than that of a randomised version of the data, see figure 6-27. This initially suggests the transmission networks are partitioned to a degree, however, the pairwise overlap between the main sources in each colony is still high shown in figure 6-29. The colonies may be able to detect that the food is all from one source close to the nest and therefore there is less need to partition the food from foragers.

The number of sources individual ants are exposed to during famine relief is higher than might be expected, see fig. 6-23, and brood workers were shown to be exposed to the same number of sources as other workers. We might expect brood workers to be exposed to fewer sources to lower the chance of passing on infections from possible pathogens to the brood. Previous studies have shown colonies taking precautions to protect the brood such as infected workers avoiding the brood chamber [58]. As we know the food used in this experiment was safe future experiments could investigate transmission pathways used when food containing a harmful substance is introduced. The ants that are exposed to the highest number of sources tend to be on average at a greater pathlength from sources, figure 6-25, i.e. at the end of long chains, which is potentially less risky than receiving directly from many sources. Direct interaction is likely to increase the chance of contracting an external parasite, such as fungal spores or mites, from a source (see Ch. 3 in [41]).

This chapter has also shown the existence and amount of transmission of stored food, 'background feeding', in all four colonies, see section 6.5. Storing food in the crops of workers is known to occur in other species, most famously in the honey pot ants which have physiologically specialised members which act as 'living storage containers', see [132]. Storing food is sensible and likely to be necessary in species which rely on food sources which tend to be ephemeral such as *T. albipennis* [111]. As this species does not readily have or build facilities inside their simple nests to store food (and perhaps because their nests are occasionally

destroyed causing emigrations [10]) an alternative solution is to store food in the living workers (which can be transported if nest is destroyed/flooded). The individuals that provide the largest amount of background food tend to be so-called 'retreaters' (external in control and internal in the famine relief treatment) and are often triggered to donate by a small reception of new food from a forager, see figures 6-31 to 6-34. This triggering stimulus is similar to that suggested by von Frisch where honey bee foragers donate a small amount of nectar to potential recruits [70]. Given that the individuals providing the largest amount of background food were potential foragers, the small donation of food from a forager may act a stimulus to either forage if the receivers crop is empty or to donate their crop if it contains stored food. Distributing background food could be a safety mechanism to dilute incoming new food or a management strategy to help get food to everyone and then help re-distribute the new food.

In addition to the general results described above, this chapter has revealed that several features of the distribution process vary between the colonies and are likely to be influenced by demographic and geometric properties as well as the number of workers. The two smaller colonies, III and VIII, provided larger amounts of food per capita compared to the larger colonies, IV and V. Colony VIII only provided a small amount of food during the control treatment which is possibly why it provides more in total and per event during the famine relief treatment, see figs. 6-6 and 6-20. Colony IV provides the least per capita which suggests that this colony had more food already stored in its workers or was less efficient at transferring large amounts to individuals. Colony IV has two main foragers providing large amounts of new food, V has many foragers each providing smaller amounts while III and VIII have similar proportions of foragers, fig. 6-19. These foragers can be thought of as 'sources' of new food. The maximum reach of these sources is high (65 to 70%) in colonies III and IV, around 50% in VIII and 35% in V, figure 6-21. This reduces dramatically in III and IV when filtered by even a small amount which potentially reduces the risk posed from exposing a high proportion of the colony to a source if the harmful substance is not effective in smaller quantities. It is interesting that the two larger colonies appear to be using almost opposite strategies to relieve the famine. Colony IV has little space between its brood pile and nest entrance to accommodate many foragers donating to rosettes of ants at once, in contrast the brood pile in colony V is very far from the nest entrance leaving plenty of space. The smaller brood pile and larvae to worker ratio in V is also likely to require many fewer individuals to care for it than the large brood in IV, this would mean many individuals in colony V could leave

the nest to forage. Future experiments would need to use standardised colonies to determine whether these strategies are consistent for the various combinations of worker number and brood size.

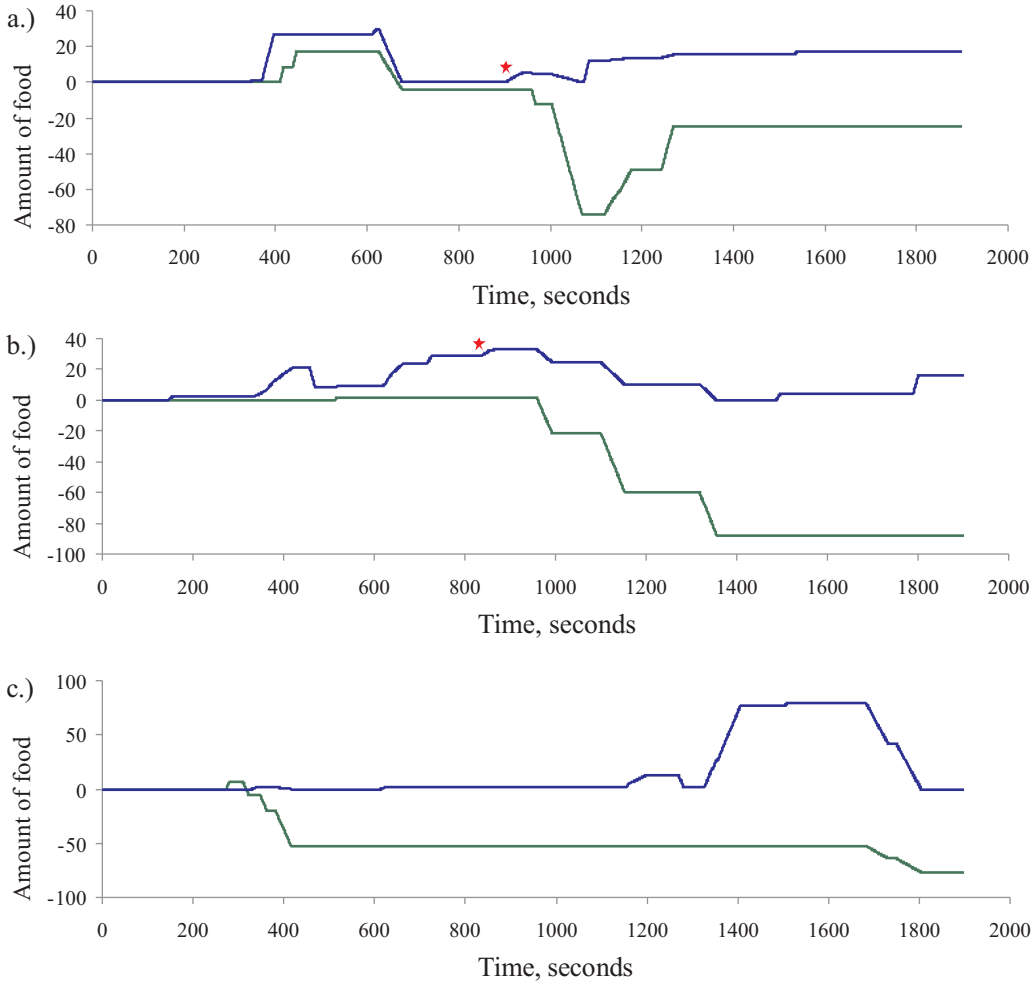

Figure 6-32: *Timelines for the largest providers of background food in colony IV, a.) ant 50, b.) ant 56, c.) ant 58. The blue line represents the net amount of new food, while the green line represents the amount of background food (which becomes negative when donated). The red asterisks indicate the small reception from an incoming forager that triggered the ant to donate their stored load.*

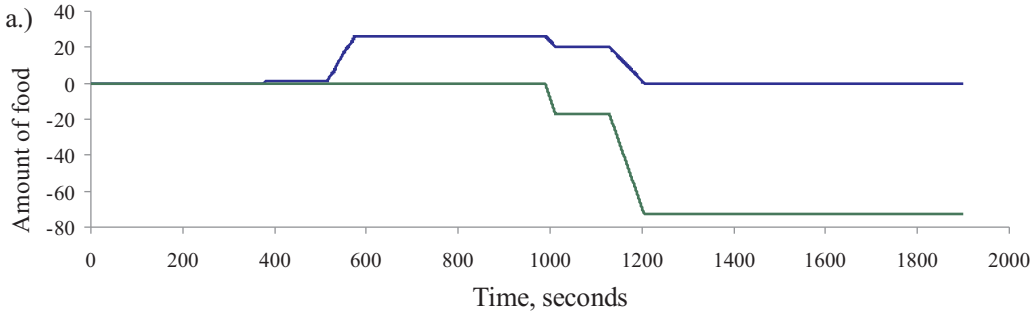

Figure 6-33: *Timeline for the largest provider of background food in colony V, a.) ant 12. The blue line represents the net amount of new food, while the green line represents the amount of background food (which becomes negative when donated). The red asterisks indicate the small reception from an incoming forager that triggered the ant to donate their stored load.*

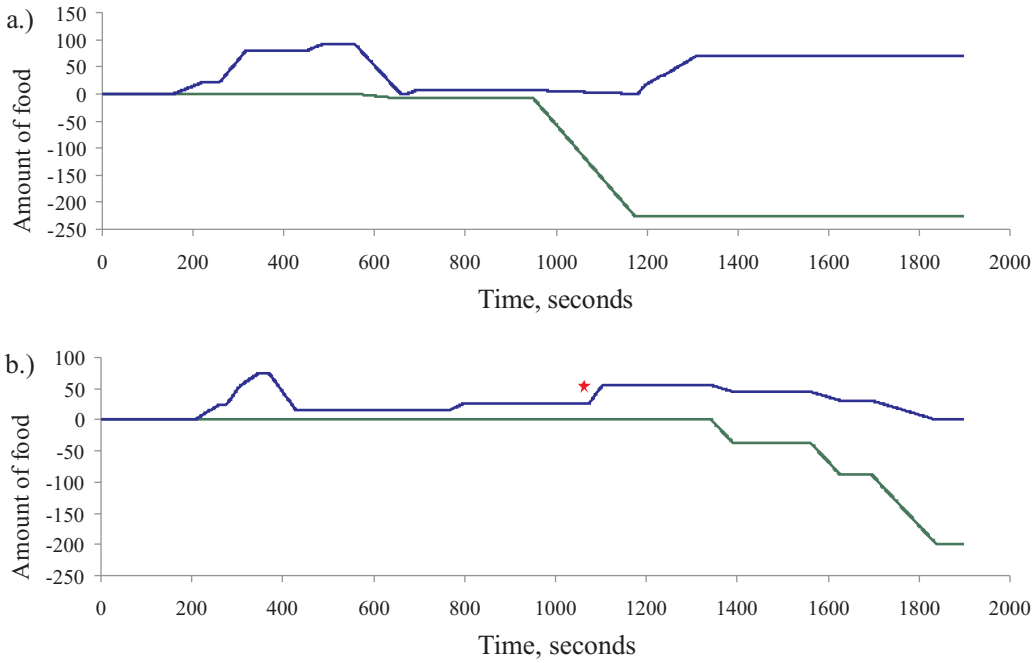

Figure 6-34: *Timelines for the largest providers of background food in colony VIII, a.) ant 5, b.) ant 9. The blue line represents the net amount of new food, while the green line represents the amount of background food (which becomes negative when donated). The red asterisks indicate the small reception from an incoming forager that triggered the ant to donate their stored load.*

Chapter 7

Conclusions

This thesis has investigated the distribution of food within four colonies of the ant *Temnothorax albipennis* under typical laboratory conditions and after a 48 hour starvation period. The aim was to see how the colonies would organise food transmission during famine relief in comparison to under normal conditions with four main objectives: A - to compare rates of feedings between the two treatments and determine how faster feeding is achieved during famine relief; B - to determine whether spatial structure is maintained during famine relief; C - to determine amounts of food distributed to individuals, whether there is an even distribution of food among the workers and whether individual role influences amount received; and D - to determine the transmission pathways used and whether they are structured in a way that might minimise or maximise the spread of a pathogen. In contrast to previous studies which have used markers in the food to trace its transmission, e.g. [73], this study has marked the workers and deduced the transmission of food from detailed observations of trophallaxis events. Using this technique the study has uncovered several previously unknown features of how this species distributes food which may have parallels in other ant or social insect species.

Chapter 1 outlined the features of eusocial insects that are relevant to this project including: features of eusociality; immunity in eusocial insects; feeding behaviour in eusocial insects; the salient features of *T. albipennis*; and the 4 objectives of this study. Chapter 2 described the materials and methods used for the experiment and the data collected from it, primarily the trophallaxis and spatial data from the two treatments. In Chapter 3 I presented the gross responses to famine

relief in comparison to under normal conditions which consequently directed the investigations in the remaining chapters. These included: frenzy behaviour; feeding in rosettes; brood coverage and increased speed of internal workers. I also described the differences in the geometric and demographic features of the four colonies. Chapter 4 investigated the food distribution process from a temporal perspective revealing that all four colonies are efficient at relieving the famine; Chapter 5 looked from a spatial perspective and revealed that the colonies alter their space use to facilitate the distribution during famine relief; while Chapter 6 investigated the amounts and pathways used to distribute food to individuals.

Objectives A and B

We have seen that, as expected, rates of feeding unfed ants were an order of magnitude higher during famine relief compared with under the control treatment. It was revealed that the rate at which unfed ants become fed follows a recovery exponential curve which is consistent with a model that assumes a well mixed system, (fig. 4-8). This result is accordant with a study on the ant *Formica fusca* which found a recovery exponential fit to the increase in the proportion of fed ants using a different technique (using scintigraphy to trace labeled food) [78]. This is a particularly interesting result in *T. albipennis* as previous studies have shown this species to have a strong spatial structure within the nest which is not a well mixed system [95, 107]. The spatial analysis in this study revealed that during famine relief this spatial structure is abandoned (sometimes to the extreme level of the queen actually abandoning the brood pile) and the groups of internal and external ants move closer together facilitating the efficient distribution of food, (figs. 3-5, 3-7 and 5-1). It is expected that the workers would eventually re-organise themselves into the original spatial structure, as seen after colony emigrations, a phenomenon known as 'social resilience' [108]. Further analysis of space use beyond the 30 minutes of the famine relief treatment would be needed to determine whether this occurs once the level of feeding activity returns to normal. Along with the spatial mixing of the workers the transmission of food during the famine relief treatment was also facilitated by donors feeding several recipients simultaneously in 'rosettes' (fig. 3-4), a behaviour reported previously in a different ant species *Solenopsis invicta* [79]. Feeding several recipients at once has the advantage of transferring food to many individuals quickly, however, it also brings individuals into close proximity with one another. This, along with internal and external ants being closer together, may have negative effects if it increases the probability of contracting an external parasite from foragers or other nest-mates (see Ch.3 in [41] and [2]). A further way the four colonies might

have created higher rates of feeding under the famine relief treatment would be to recruit a large number of foragers. However, the spatial analysis revealed that more ants remained inside the nest during the famine relief treatment compared to under the control, (table 2.3 and fig. 3-7). The function of such seemingly counter-intuitive behaviour may be to facilitate the transmission inside the nest once the foragers had introduced the food as proposed in [88] which also found a higher proportion of ants inside the nest after the re-introduction of food. We have seen that instead of increasing the number of foragers, individuals increased their foraging effort by making a higher number of trips outside the nest during the famine relief treatment, (fig. 5-13). This is similar to what has been found in other studies, e.g. in honey bees [65], but is contradictory to what is expected given the foraging behaviour of *T. albipennis* in the wild. Scavengers are expected to develop mechanisms to create a rapid concentration of nest-mates at a new food item, i.e. to recruit lots of foragers, to prevent losing it through competition [111]. Perhaps the proximity of the food source to the nest in this case meant that it was not necessary to recruit many foragers. Other factors causing potential foragers to remain inside the nest were discovered through careful analysis of the amounts of food distributed for objective C.

Objective C

Previous studies have suggested that the duration of a trophallactic interaction is related to the amount of food transferred [117, 37]. The recorded durations of the trophallaxis events in this study have allowed estimates for the amount of food provided in total, the net-food of each individual and the amount transmitted per event to be calculated. Experimental work is required to test the relationship between duration and volume of food transmitted in trophallaxis and determine more precisely the relationship between the amount transferred and the number of recipients feeding simultaneously. Experiments could also test whether the rate of transfer of food varies as a function of colony size or hunger. The anaylsis of the amounts transmitted revealed several interesting features and led to the discovery that several ants in each colony had food stored from before the starvation period which they later donated, (fig. 6-30 and figs. 6-31 to 6-34). This is a feature that could not have been discovered had only a sample of the feeding interactions been used as it would not be possible to determine whether the large amount of background food an individual donated actually originated from the new food source, i.e. whether a large donation to the individual from a forager was not included in the sample. These donations of background food were often triggered by a small reception of new food from a forager. von Frisch proposed

that recruitment in honey bees might involve foragers donating a small amount of food to potential recruits [70]. Perhaps the same stimulus is triggering these ants to donate their stored food particularly as the largest providers of background food were foragers during the control treatment so potentially could forage during famine relief if their crops were empty. Storing food for times of need is a sensible precaution to take and occurs in species which have the ability to physically store food inside the nest, e.g. in 'cells' in honey bees. Storing food inside crops of individuals for later consumption by others is known to occur in other species most famously in *Myrmecocystus* ants where members of the honeypot caste in a colony act as specialised 'living storage containers' , see [132]. However, this is the first time to my knowledge that the distribution of this stored food has been shown to occur alongside the distribution of new food. The purpose of such dual transmission may be to reduce the toxicity of any undetected harmful substances in the new food by mixing with old food. This may be an adaption to counter the fact that foragers are not always able to detect harmful substances in the food they collect [55]. Alternatively the purpose may be to allow the individuals storing the food to facilitate the distribution of both types of food allowing active foragers to return to the food source more quickly and eventually enabling these individuals to return to foraging themselves once their crops are empty.

The distribution of net-food received by individuals showed that there is an uneven distribution of food within the colonies with some individuals receiving a relatively large amount, (fig. 6-7). Wilson and Eisner proposed from their study that the gut content of the colony would tend to uniformity over time, [73], whereas other studies found an uneven distribution among workers, e.g. [78, 80]. The results from this study found an uneven distribution of food among workers, however, looking beyond the 30 minutes of the famine relief treatment may reveal that gut content does tend to uniformity over a longer time period. Similar to findings by Buffin et al., [78], this study found that the potential storage capacity of the colony was not reached. Without dissecting the workers it is difficult to know how much food they have stored in their crops; perhaps capacity is not reached because workers already contain food. In addition, it may not be beneficial to fill all the workers with one food type; conceivably some storage capacity is left available to store other food types that are required by the larvae and queen.

The categories determined from individual space use revealed that the brood ants tended to have the larger amounts of net-food by the end of the 30 minutes. This is perhaps surprising given that a study using honeybees found that individuals

which care for the brood received the least food compared with other task groups, see [80]. However, nurse bees in honeybee colonies mainly utilize protein from pollen while the food provided in the experiment was sugar syrup a carbohydrate source. Perhaps in this case the brood ants were hungriest because they had been using up their own reserves to feed the larvae during the starvation period and required the most food to continue doing so. Alternatively, they may have been taking on large amounts of food in order to act as living reserve stores. A previous study in *T. albipennis* found that some individuals act as 'repletes' storing more lipids than others during the colder seasons [23]. Perhaps these individuals were taking on a similar role (but for sugars not lipids) given that the experiments were conducted at the start of winter and the colonies are likely to be storing food. Without following these individuals beyond the 30 minutes of the famine relief treatment it is not possible to say for definite the purpose of transmitting a large amount to certain individuals. However, we can postulate that the purpose is either: to store large amounts for times of need (like the ants which had stored background food in this study or those storing lipids in other studies [23]); to test the safety of the food; or, alternatively, to distribute the large quantity within the nest enabling the foragers to return to the food source more quickly.

The feeding data showed that a large proportion of ants in each colony received food more than once, (section 4.10 and fig. 6-9). The purpose of this may be to facilitate the rapid transmission of a small amount of food to all individuals then to 'top-up' that initial amount later with subsequent feedings (Objectives A and C). A previous study has shown that a uniform distribution of food among fire ant larvae is achieved by nurses feeding them in many small donations [64]. In the current study the final distribution of food among workers was not uniform (for the 30 minute period analysed). However, feeding in multiple events may be a way to ensure that all individuals get at least the minimum amount of food, without a need for centralised control, while at the same time informing the foragers of the hunger level of the colony through a high number of interactions with different individuals. Only giving most ants a small amount of food at once could also be a risk management strategy so they can mix food from different foragers and from background food providers to dilute any harmful substances that may be in the new food.

Objective D

This study verified the existence of 'chains of transmission' during food distribution but also went on to explore these pathways in terms of how a pathogen

or parasite might spread through them. A study by Otterstatter and Thomson showed that the characteristics of the contact structure in a eusocial insect colony, namely the contact rate, determines infection rate by a pathogen [38]. At first the transmission networks of new food during the famine relief treatment appear very tangled and without structure, (figs. 6-11 to 6-14), suggesting a pathogen could spread rapidly to all the workers. On careful analysis this study has shown that the networks are more partitioned than a network with recipients selected at random, (fig. 6-27), suggesting some measures are taken to reduce pathogen spread. However, between foragers that feed a large proportion of the colony there is still a high level of pairwise overlap in the ants that their food reaches, (fig. 6-29). In this study only one source of food was provided and was placed fairly close to the nest entrance. This factor may explain why a high level of overlap exists between foragers which feed a large proportion of the colony if they knew that there was only one food source and there was therefore no reason to strictly partition who they feed. In addition to this, *T. albipennis* nests tend to only have a single chamber; other species which have more spatially segregated nests may be expected to show stronger partitioning, for example leaf cutter ants [52]. However, we do know that *T. albipennis* shows seasonal polydomy [10]. It is thought that the reason for this is to create more nest space during the warmer seasons when activity is higher. This seasonal polydomy could also be a strategy to spatially segregate the colony to improve chances of survival. One of the features found from previous network studies in social insects was a higher heterogeneity in contact structure with increasing colony size [59]. In this study the largest colony, IV, showed the highest heterogeneity with two foragers responsible for feeding most of the colony. This may occur because under certain circumstances larger colonies are more likely to have specialists, [133], but also perhaps because this large colony had the largest brood pile which would require many brood workers and therefore could not spare many workers for foraging. This is actually contrary to previous findings in this species that larger colonies do not have more specialized workers than smaller colonies [129]. However, while the brood to worker ratio for the large colonies used in [129] was much higher than those used in this study, the number of larvae is not given. This ratio may be more important in determining the amount of specialisation in larger colonies and this study effectively only has a sample size of one for large colonies with a high larvae to worker ratio.

One of the assets of this study is the sample size in comparison to that of similar studies. Typically only one or two colonies of a species are used in studies of

food transmission in ants, for example see [73]. This study has uncovered several differences among the four colonies in terms of how they relieve the famine which are described in the summary sections of the main results chapters. These differences highlight the flexibility of ant colonies to respond to a problem, the variety in strategy potentially promotes robustness within the species. The study has demonstrated that colony size, i.e. number of adult workers, is not the only factor that can influence the organisation of food transmission. Brood properties and the amount of food stored in the workers inside the nest also potentially effected the distribution process. This is evident from the very different strategies employed to relieve the famine by the two large colonies, IV and V, in particular from the number of foragers employed. IV only used two primary foragers to do most of the provisioning whereas colony V used nearly 30. V has a much lower larvae to worker ratio meaning the colony can probably spare more individuals to forage whereas colony IV, which has a much higher larvae to worker ratio, is likely to require many brood workers. Meanwhile, the amount of background feeding was highest in colonies IV and III. Colony VIII provided the least food during the control treatment which probably explains why there was so little background feeding in this colony (having not stored much during the control treatment) and why VIII provided most new food during famine relief. The size of the brood pile is likely to have affected the speed at which workers moved. Colony IV had the largest brood pile and the slowest moving workers whereas colony V had a small brood pile far away from the entrance and had the fastest moving workers. This constraint on the movement of workers is likely to have influenced how fast the famine was relieved and contributed to colony V being the most efficient at first feedings (fig. 4-8).

It is plain to see that if only one of these colonies had been studied, while it would have been much quicker to analyse, we would get a biased perspective on how this species organises feeding during famine relief. The disparity in behaviour between the four colonies reminds us that ants are excellent problem solvers and that due to the different demographic and geometric properties the most suitable solution may not necessarily be identical in each case. The next step for future work would be to carry out the experiment on standardised colonies, i.e. manipulate the number of brood items and their location inside the nest, to control for the effects of the brood pile. As an example, we might hypothesise that large colonies with a big brood pile (and high larvae:worker) will employ fewer foragers as evidence from this study and others suggests, e.g. [59]. In some aspects using four very different colonies in this study might have been a drawback in terms

of sample size (i.e. n=1 for a large colony with high larvae:worker), however, it provides a good starting point for exploring resource distribution in ant colonies and highlights the different ways colonies can respond to a situation which might not have been realised had standardised colonies been used from the outset.

The work done in this study can be seen as part of a much larger project which concerns the distribution of resources within ant colonies. There are many features that were not approached in this study and would be appropriate for future investigation, these include: grooming networks, to see if preferred grooming partners are also feeding partners; behaviour during the starvation period, to see how much background food is distributed in this time; using longer starvation periods repeated on the same colony to see if their famine relief response changes as the nutritional needs of the colony increases; whether colonies partition their transmission networks more strongly when there is more than one food source at different positions outside the nest; worker-brood interactions; and responses to food sources that are actually contaminated with something harmful, for example with alcohol, to represent a poison, or a pathogen such as the entomopathogenic fungus *Metarhizium anisopliae*. Once we know more about the process we can begin to develop models which describe how colonies might distribute resources under different conditions. Clearly the rate-limiting step in this study has been the manual tracking of individual ants. In total 252 and 250 ants were tracked in the control and famine relief treatments respectively with 1024 trophallaxis events recorded from the two treatments (110 during the control and 914 during the famine relief treatment). There is much interest and need for progress in the field of automated tracking which has driven recent developments in this area (for example see [114]). It is therefore likely that these techniques will be further developed in the near future which will enable more studies in food distribution to be conducted at a faster rate. The discoveries made in this study are examples of the previously unknown features of the distribution process inside the nest of colonies that can only be revealed through knowing the detailed behaviour of all individuals. Automated tracking will enable much more research to be done in this area and resolve some of the many aspects that remain a mystery.

Appendices

Appendix A

First Feeding Curve Fitting

Colony	Treatment	R^2	F	Sig.	Constant	b1	b2
V	Control	0.879	101.803	<0.005	0.758	-0.018	0.001
VIII	Control	0.434	10.735	<0.005	0.777	-0.12	0.0004
V	Famine Relief	0.874	97.314	<0.005	0.569	0.029	-0.001

Table A.1: Quadratic fits to the proportion of tracked ants inside the nest as a function of time

Colony	Treatment	Gradient	SE for gradient	R^2	P for A-D normality test for residuals
III	Control	0.12948	0.01520	81.9	0.568*
IV	Control	0.10259	0.00839	76.9	<0.005
V	Control	0.13071	0.00591	91.6	<0.005
VIII	Control	0.12236	0.01697	77.6	0.178*
III	Famine Relief	0.56694	0.02701	91.9	0.034*
IV	Famine Relief	0.37609	0.00674	97.3	0.441*
V	Famine Relief	0.69619	0.01750	95.7	0.023*
VIII	Famine Relief	0.39776	0.01026	97.2	0.720*

Table A.2: Gradients of logarithmic fits to first feeding curves. Asterisks indicate data set is normal or near normal.

Colony	Treatment	Gradient	SE for gradient	R^2	P for A-D normality test for residuals
III	Control	261.90	28.74	83.8	0.959*
IV	Control	37.01	4.02	65.3	0.029*
V	Control	42.87	4.05	71.3	0.189*
VIII	Control	258.83	35.52	78.0	0.978*
III	Famine Relief	876.44	50.99	88.3	<0.005
IV	Famine Relief	352.74	15.11	86.5	<0.005
V	Famine Relief	674.79	34.65	84.2	<0.005
VIII	Famine Relief	450.25	22.82	90.1	<0.005

Table A.3: Gradients of Michaelis-Menten fits to first feeding curves. Asterisks indicate data set is normal or near normal.

Colony	Treatment	Gradient	SE for gradient	R^2	P for A-D normality test for residuals
III	Control	0.180	0.017	87.5	0.212*
IV	Control	1.212	0.067	87.9	0.012*
V	Control	1.153	0.022	98.4	0.007
VIII	Control	0.200	0.024	82.7	0.581*
III	Famine Relief	1.341	0.096	83.3	0.013*
IV	Famine Relief	5.563	0.190	91.0	<0.005
V	Famine Relief	9.996	0.279	94.8	<0.005
VIII	Famine Relief	1.427	0.054	94.1	0.766*

Table A.4: Gradients of square-root function fits to first feeding curves. Asterisks indicate data set is normal or near normal.

Colony	Treatment	Gradient	SE for gradient	R^2	P for A-D normality test for residuals
III	Control	-1439.4	311.7	57.1	0.277*
IV	Control	-338.1	88.53	29.9	0.055*
V	Control	-469.9	94.02	35.7	0.021*
VIII	Control	-1327.3	336.5	50.9	0.711*
III	Famine Relief	-12394.9	487.4	94.3	0.063*
IV	Famine Relief	-10031.3	296.3	93.1	<0.005
V	Famine Relief	-17006.3	214.8	98.9	0.534*
VIII	Famine Relief	-6866.6	254.1	94.4	0.457*

Table A.5: Gradients of inverse function fits to first feeding curves. Asterisks indicate data set is normal or near normal.

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